Doctoral Dissertation

Doctoral Program in Bioengineering and Medical-Surgical Sciences
(32th Cycle)

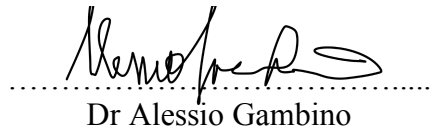
Potential use of Optical Coherence Tomography in patients with Oral Lichen Planus treated with photobiomodulation or standard medication.

Candidate
dr. Alessio Gambino
* * * * * *

Supervisors
Prof. Stefano Carossa
Prof. Paolo G. Arduino, Co-Supervisor

Doctoral Examination Committee:
Prof. Marco Meleti , Referee, University of Parma
Prof. Umberto Romeo, Referee, University of Rome La Sapienza

I hereby declare that, the contents and organisation of this dissertation constitute my own original work and does not compromise in any way the rights of third parties, including those relating to the security of personal data.



Dr Alessio Gambino

Summary

Introduction. Oral lichen planus (OLP) is a relatively common chronic inflammatory disease (1-2% of the total population), of unknown aetiology. To date, the gold-standard treatment remains high-potency topical corticosteroids. In oral medicine, clinical studies have demonstrated the effectiveness of PBM in patients with OLP, whenever poorly responsive to first-line approaches. Optical Coherence Tomography (OCT) could repeat, in real time, an image of architecture of epithelial and sub-epithelial tissues and surrounding structures. To date, OCT has been scarcely used in oral medicine, with no study focusing on ultrastructural changes in patients with OLP undergoing different treatments.
AIMS: **AIM 1**: to compare OCT scan of healthy mucosa and of atrophic-erosive OLP to the traditional histopathology, in order to create a preliminary pattern between pathologist and clinician. **AIM 2**: to evaluate, through OCT, the morphometric changes of the oral tissues of patients with erosive and painful OLP that have performed the topical steroid therapy (Group A), compared to Photobiomodulation- PBM (Group B).
Materials and Methods.
Patients: Two groups (Group A and B) of 20 patients each, selected from a random original sample of 100 patients affected by erosive and painful OLP, referred to the Department of Oral Medicine, CIR Dental School, Turin untreated in the previous eight weeks.
Optical coherence tomography: a recent variant (OCT oral instrument: a variant (OCT oral instrument, version 2.1) of a commercial frequency domain swept source OCT dermatological instrument (SS-OCT, VivoSight® Michelson Diagnostics Ltd, version 2.0, Orpington, Kent, UK) was deployed. Length of the probe was 124 mm, probe shaft diameter was 15 mm; field of view of 6 mm^2. OCT (enface and dynamic) scans were obtained before and after treatment, and six months after the end of the eight-weeks treatment.
Study design: Group A would undergo a eight weeks “gold-standard” treatment with two daily application of clobetasol dipropionate 0.05% in an aqueous gel of 4% hydroxyethyl cellulose (100 g) in equal parts (50:50). Group B would undergo eight PBM sessions – once a week for eight weeks - with “Raffaello diode laser" 980/645 nm, used with the following parameters: output power = 300 mW, power density =

1 W/cm^2, fluence = 4 J/cm^2, collimated probe of 0.6 cm in diameter and spot size of 0.28 cm^2, kept perpendicularly at 2 mm from the area of irradiation. A "spot" technique with a slight overlapping would be carried out in each site, in order to distribute energy evenly on the mucosal lesions and the peri-lesional tissues up to 0.5 cm.

Results. AIM 1: the following OCT features were commonly registered among the patients affected by erosive OLP belonging to both groups: epithelium (EP) at enface scan revealed less width, and higher hyper-reflectiveness than EP of a healthy mucosa, indicative of either hyperkeratosis or hyperparakeratosis. At dynamic scans EP scattered red dots emerged within EP, which might be attributed to the concurrent intra and inter-cellular oedema, as expected in cases of acanthosis and spongiosis, commonly encountered in OLP. Lamina Propria (LP) revealed loss of integrity and hyper-reflectiveness at enface scans, with an increased, denser red pattern of vascularization at dynamic scans.

AIM 2: EP and LP width showed significant fluctuation after treatment, both in Group A and Group B. Specifically, paired t-student test showed a significant increase of EP width ($p < 0.01$) for both groups after eight-weeks treatment, indicative of a partial healing of the atrophic epithelium. On the other hand, paired t-student test displayed a significant decrease ($p < 0.01$) of LP width in both groups, indicative of a possible reduction of the activity of the band-like inflammatory infiltrate. Unpaired t-student test revealed a significantly higher increase of EP width in Group A, when compared to Group B ($p < 0.01$), at the end of treatment; on the contrary, no significant decrease of LP was detected between the two groups ($p > 0.05$). After six months, these variations were not preserved, with LP and EP width in both groups returning to be not significantly different from pre-therapy pattern ($p > 0.05$).

Conclusions. This is the first project that would analyze and show significant ultrastructural changes of the oral mucosa after PBM and clobetasol with OCT. No significant differences were found between the two groups. The main limitation of this study is the operator-dependent approach required for an innovative technique, since no probe for a thorough analysis of the oral cavity has been standardized yet. Although biopsy remains the gold standard for OLP in oral medicine, OCT seemed to be a helpful tool for the clinician and the pathologist. Further studies on larger samples are needed, ideally with a designated probe for the necessities of the oral physician. Further clinical entities, such as other premalignant disorders, or autoimmune bullous-erosive diseases requiring constant follow-up or/and therapy, should be investigated, to fully understand the true scope of action of OCT in oral medicine.

Acknowledgments

I would like to acknowledge Mr Jon Holmes – CEO for Michelson Diagnostics Ltd, for the precious collaboration to the shipment of the Device needful for this project.
Dr. Liam Sexton, the OCT Applications Engineer for the training and supporting during the first tryouts of the device.
Mr. Colin Hopper, for his experience and helpful collaboration during the experimental phases of this project.
Dr. Adam Strange for the demonstration on data collection and processing of OCT application.

This work is the result of an agreement of collaboration between UNIVERSITY OF TURIN – DEPARTMENT OF SURGICAL SCIENCES – CIR DENTAL SCHOOL and UCL EASTMAN DENTAL INSTITUTE, DEPARTMENT OF MAXILLOFACIAL MEDICINE & SURGERY LONDON UK.
Therefore I would like to thank the Director of Eastman Institute prof. Stephen Porter, for the loan of the Device on which I have prepared my research.

Prof. Stefano Carossa, my Tutor, for his personal supervision and for encouraging my researches on this new and innovative device.

Prof. Elio Berutti, Director of CIR Dental School, for allowing the realization of these researches within the Dental School of Turin.

Administrative and Secretarial staff Mrs. Antonella Davello and Mrs. Nadia Gardone, for their precious collaboration in the difficult bureaucratic phase of communication between the various offices in charge.

I would also like to thank my colleagues with whom I have had the pleasure of working for more than ten years.

Firstly, prof. Paolo Arduino, my PhD co-supervisor, for having once again trusted my ideas and me.

Prof. Roberto Broccoletti for allowing the realization of this work within the Oral Medicine Unit of CIR Dental School of Turin.

Dr. Adriana Cafaro and dr Ercole Romagnoli, whose ideas gave birth to this research project and its subsequent developement.

Dr. Marco Cabras, for his friendship and professionalism, and for being by my side especially in the darkest moments of this course of study.

Dr. Luigi Chiusa, for his collaboration in the descriptive and laboratory part and dr. Melania Lupatelli for her collaboration in interpretation of images.

Dr. Mario Carbone, Dr. Davide Conrotto and Dr. Paola Carcieri for the constant interest, and for the helpful participation during my journey and on this project.

Prof. Umberto Romeo and prof. Marco Meleti for agreeing to review this work.

Thanks to my youngest workmates (Marco, Nicolò, Dora, Silvia, Giuliana and Ludovica) for their motivation and for making my work in the Oral Medicine Unit easier and more enjoyable.

Finally, thanks to my phd-mates : dr Francesca Serra and dr Gabriele Rossini : this PhD program without you it would not have been the same…I'll miss you!!!

It was a great honor and privilege to work and to collaborate with each one of you for this international project; my hopes for the future are to continue in this path of friendly and constructive collaboration, just as
I did in these last years, both in the field of oral medicine and, more generally, in dentistry.

I would like to dedicate this thesis to my best love and loving parents. Thank you for always being close to me, not abandoning me in difficult times and for always understanding me patiently, despite my mood can be unfathomable at times.

Contents

SECTION 1

Chapter 1

Chapter 2

Chapter 3

SECTION 2

SECTION 1

INTRODUCTION

Chapter 1

1.1 Oral Lichen Planus

Oral lichen planus (OLP), is a common chronic inflammatory disease of disimmune origin triggered by genetic malfunction and/or environmental factors of unknown etiology. OLP commonly arises in the fourth decade of life, affecting 1–2% of the population, with a female-male ratio of 4:1 (1).
Clinically, OLP is commonly grouped in two clinical forms: one with only white lesions, presenting as white striations, plaques or papules, whilst the other arises with red, atrophic-erosive lesions, with or without concomitant reticular lesions. (2)
Buccal mucosa is the most commonly affected oral site in both white and red OLP, usually with symmetrical involvement of the posterior third of both sides, followed by tongue and gingiva; less frequently, lip, hard and soft palate, floor of mouth might be involved. (3)
The diagnosis of reticular lichen planus is usually based on the clinical findings alone. Interlacing white striae appearing bilaterally on the posterior buccal mucosa in patients without history of bone marrow transplant (who could simulate oral GvHD) is often pathognomonic.
Erosive or atrophic types should be differentiated from bullous diseases as both may have a desquamative clinical appearance. Nevertheless, bullous diseases occurs with erythematous lesions or blatant ulcers with no surrounding white striae; furthermore, epithelial desquamation might occur after a slight pressure is applied on an unaffected area (Nikolsky's sign), especially in case of gum involvement, thus helping differentiation from erosive and erythematous OLP. (4)
The clinical pathway of OLP concern two aspects: the potential risk of malignant transformation, and the propensity for persistence or/and relapse of atrophy, erosions, and the concurrent symptoms, regardless of therapy (5,6).

1.2 Pathological features of Oral Lichen Planus

OLP is a T-cell mediated autoimmune disease in which CD8+ T cells trigger apoptosis of the basal cells of the oral epithelium. Both CD8+ and CD4+ cells migrate into the epithelium either due to random encounter of antigen during

routine surveillance or due to a chemokine-mediated migration toward basal keratinocytes.
Apoptosis of keratinocytes can jeopardize the normal integrity of the basement membrane (BM), which is maintained by a living basal keratinocyte due to its secretion of collagen 4 and laminin 5 into the epithelial basement membrane. At the same time, a non-intact basement membrane cannot send a cell survival signal, thus triggering apoptosis, and setting a vicious cycle, which relates to the chronic nature of the disease. (7)
A similar vicious cycle involves lymphocytes, with CD8+ cells being activated by antigen binding to major histocompatibility complex (MHC)-1 on keratinocyte or through activated CD4+ lymphocytes. The activated CD8+ T cells destroy the basal keratinocytes through tumor necrosis factor (TNF)-α. Subsequent antigen presentation to CD4+ cells and Interleukin (IL)-12 activate CD4+ T helper cells, which activate CD8+ T cells (8).
OLP histological pattern is characterized by a band-like subepithelial lymphocytic infiltrate, presence of intraepithelial lymphocytes with interface lesion. Lymphocytes are the predominant cells, whereas plasma cells can be strongly associated with deep extension of the inflammatory process as well as with epithelial erosions. If band-like inflammation and interface vacuolar alteration of the basal layer are considered specific features for OLP diagnosis, while other accompanying features, such as parakeratosis, acanthosis, Civatte bodies or fibrinoid deposits along BM are considered as “nonspecific”(4).

1.3 Treatment of Oral Lichen planus

Treatment of OLP is difficult and aimed at palliation rather than cure, and aimed towards symptomatic patients who suffer from painful anthropic-erosive OLP.
At present, the treatment most commonly suggested involves the administration of corticosteroids. On the other hand, others drugs like calcineurin inhibitors, retinoids, dapsone, hydroxychloroquine, mycophenolate mofetil and enoxaparin have contributed significantly in cases unresponsive to corticosteroids (10,11,12,13).
The topic corticosteroids drugs are used for their ability to modulate inflammation and immune response. They act by reducing the lymphocytic exudate and stabilizing the lysosomal membrane (14). Of these, clobetasol has been widely reported to be effective in the treatment of OLP lesions through its prevention of inflammatory processes such as oedema, fibrin deposition, vasodilation, and phagocytic activity (15). According to a recent systematic review, topical application of 0.025 or 0.05% clobetasol propionate should be considered the first therapeutic option in the management of erosive OLP (16).
The greatest disadvantage in using topical corticosteroids is their lack of adhesion to the mucosa, due to the constant salivary ashout to wich the mouth is commonly exposed, throughout the day. Although topical steroids along with adhesive base (i.e, (carboxymethyl cellulose, hydroxyethylcellulose) have been experimented in trials, no study showed their superiority when compared to steroids alone (10).

Systemic corticosteroids are reserved for erosive or erythematous OLP recalcitrant to topical approaches or widespread in the whole oral cavity (13).
Nevertheless, there are other therapeutic choices for localized painful erosions. In fact, literature offers increasing evidence of alternative non-pharmacological modalities.
Among this relatively new treatments are counted photochemotherapy with 8-methoxypsoralen and long wave ultraviolet light (PUVA), Photodynamic therapy (PDT), and Laser Therapy. Concerning the latter, different types of laser have also been tested: CO_2 laser, low-dose excimer 308-nm laser and low level laser therapy also called photobiomodulation (PBM) (17,18).
Overall, palliation can be achieved in a majority of cases through topical application of corticosteroids, with or without the combination of other immunomodulators. Rarely does the condition necessitate systemic therapy. Laser therapy and other recent modalities are tried as the final remedy but their effectiveness is yet to be proven (16).

Chapter 2

2.1 Laser Photobiomodulation (PBM)

Nowadays, progress made great advances in the field of dentistry, with new techniques, surgical procedures and protocols repeated and renewed. The current diagnostic capabilities and therapeutic dentistry seem endless, thanks to the discoveries that refine new techniques.
The laser is a high-tech tool used in various fields of civil life industrial, commercial, and in the areas of telecommunications. Laser in medicine areas has a great development and Dentistry could not stay out of this challenge. Over the past 40 years, the use of lasers in oral and maxillofacial surgery has changed the surgical techniques. The fields of application of laser energy were represented by the structural abnormalities of the temporomandibular joints, pre-cancerous lesions of the oral cavity, the implant prosthesis and post-traumatic facial skin lesions; especially oral surgery makes use of surgical lasers such as diode, CO_2, erbium, neodymium, and with the evolution of technology, the use of the laser becomes more efficient by minimizing the invasiveness and discomfort of the patients.
The main biological chromophores are water, haemoglobin, melanin and the hydroxyapatite and the behaviour of the different wavelengths determines absorption spectra, which allow predicting the effects on the tissues. The photochemical effects are the basis of the principles of laser photobiomodulation (PBM). This term suggests a therapeutic approach based on the use of low-intensity laser or light-emitting diodes with the aim to stimulate a cellular function:

- anti-inflammatory effect (increase the speed of the microcirculation, reduction interstitial fluid, selective action on the lymphatic drainage of the terminals).
- biostimulation effects (a series of biological reactions that stimulate the regenerative properties and healing of tissues, non-invasively, without side effects and reducing the pharmacological support).
- analgesic effect, divided into: INDIRECT: secondary anti-inflammatory effect that produced the reduction of edema, tissue hypoxia and noxious stimuli; and DIRECT: hyperpolarization of the membrane of the nerve fiber with selective closure of the channels sodium/potassium.
- antibacterial effect (laser radiation with an appropriate wavelength, acts directly on the bacterial cell) (19)

PBM consists on the application of light with the aim of encouraging tissue healing, reduce inflammatory pathways, delivering analgesic effects with no blatant temperature rise within the tissue exposed to such approach and, as a consequence, no significant change in the tissue architecture. (20, 21)

PBM therapy relies on the usage of light in the red or near-infrared (NIR) region, with wavelengths ranging from 600-700 to 780-1100 nm; it also refers to the LEDs laser, usually provided with a power density from 5 mW/cm2 to 5 W/cm^2.

As previously mentioned, the irradiation can be delivered either with a continuous wave or with a pulsed light; although the low-density beam (0.04 to 50 J/cm2) the output power can range from 1 mW to 500 mW,. (22)

2.2 PBM in dentistry.

PBM has been experimented as a novel treatment for dental common disorders such as dental hypersensitivity, with only in-office subgroups treated with chemical or physical tubular occlusion and nerve desensitization showing statistically significant difference from placebo and in-home chemical and physical tubular occlusion showing significant difference with placebo. (23)

In retreatment of periapical lesions, PBM does not seem to prevent postoperative pain with only one high-risk study included in Cochrane, showing no differences against placebo group (very low quality evidence). (24)

Regarding periodontal diseases, PBM does not seem to offer a significant reduction in the expression of pro-inflammatory cytokines in the gingival crevicular fluid of patients with chronic periodontitis, just as other form of laser therapies (high-intensity, antimicrobial photodynamic therapy) investigated also in this review. (25)

Specific diode lasers with scaling/root planning in non-surgical periodontal therapy do not seem to have any significant effect, when compared to SRP alone, on probing depth (PD), clinical attachment loss (CAL), plaque scores (PS), with just a small but significant effect on bleeding scores (BS) and gingival index (GI). (26)

PBM seems to offer clinical advantages in terms of width of keratinized tissue and 1-year follow-up of PD and CAL in patients undergoing laser and surgical

treatment of gingival recession with flap graft technique, when compared to surgery alone, showing no benefit on root coverage and esthetics. (27)
In patients with iatrogenic inferior alveolar and lingual nerve injury after mandibular third molar surgery, PBM does not seem to provide significant improvement in sensation.
Seven trials collected overall by three systematic reviews on this subject show low-to-very-low evidence available. The main reasons reside on the heterogeneity of intervention, risk of bias of the trials selected and outcome assessment; (28,29)
Current evidence shows laser therapy in combination with surgical/non-surgical therapy provided minimal benefit in PD reduction, CAL gain, amount of REC improvement, and PI reduction in the treatment of peri-implant diseases. Lasers when used as an adjunct to non-surgical therapy might result in more BOP reduction in the short term. However, current evidence allowed for analysis of only Er:YAG, CO_2, and diode lasers. Studies on others failed to have controlled evidence supporting their evaluation. (30)

2.3 PBM and Oral Medicine

PBM seems to be a safe and effective treatment alternative for the management of recurrent herpes labialis and recurrent aphthous stomatitis although the great variety of parameters involved – 632.5-870 nm range for wavelengths, 5-80 W range for power output, 2.04-48 J/cm^2 range for power density – warrants the necessity for better designed RCTs with standardized laser protocols. (31)
PBM seems to be effective also in prophylactic treatment of radiotherapy-induced oral mucositis for patients undergoing radiotherapy for head and neck cancers, being more effective, especially on grade 0-2 oral mucositis, with the following parameters as most reliable: 633-685 nm to 780-850 nm being wavelengths, energy density of 10-150 mW, dose of 2-3J/cm^2 up no further than 6 J/cm^2, pulsed emission type (<100 Hz), for a total of two-three times a week, up to a daily dosage.(32)
In burning mouth syndrome the literature seems to highlight a possible role for PBM in reducing subjective pain in burning mouth syndrome, although the great variety of parameters involved – 630–980m wavelength, 20–300 mW power output, 0.53–176 J/cm^2 energy density of laser, 10 seconds to 15 min exposure time, one to 20 laser sessions - underlines the need for more clinical trials.(33)
PBM seems to be associated with superior outcomes in the management of bisphosphonate-related osteonecrosis of the jaw (BRONJ), with laser-including treatment being associated with superior outcomes in terms of cure or improvement of BRONJ, when compared with conventional surgical and/or conservative drug therapy. Minimally invasive surgery and PBM could be the gold-standard in early stages of BRONJ.(34)
The study of Yousef et al (35) showed that PBM is efficient in the treatment of recalcitrant oral lesions pemphigus simultaneously with conventional therapy especially in patients who do not respond to conventional treatment, according to the poor literature.(36)

For the treatment of Mucous Membrane Pemphigoids some cases series evaluated the potential application of PBM as a promising alternative to steroids or immunomodulate therapy. (37,38)
Finally, in the management of symptomatic oral lichen planus, the statement of the authors highlight that the transformation of erosive lesions to atrophic or reticular types is of valuable benefit in OLP affected patients as it can reduce painful symptoms. (39) Although the great variety of parameters involved – 630-980 nm, range for wavelengths, 20-300 mW range for power output, and 10 seconds to 15 minutes range for time of irradiation – suggests the urge for more RCTs on larger samples. (40)

Chapter 3

3.1 The Optical Coherence Tomography

Optical Coherence Tomography (OCT) is a non-invasive imaging technique which uses low-coherence interferometry to generate cross-sectional images of the architecture of a tissue. Typically, OCT relies upon a layout similar to Michelson interferometry, in which light is divided into two arms: a reference arm and a sample arm. Light in the reference arm is reflected from a mirror to a 2x2 fiber coupler, where it intertwines with the backscattered light coming from the sample under scrutiny, through the sample arm. Such a combination leads to the formation of an interference pattern, which allows calculation of the depth reflectivity pattern of the sample and its conversion into a two-dimensional, high resolution image. (41)
OCT is a cross-sectional imaging technique that is applicable to in-vivo medical examination. The technique is analogous to ultra-sound scanning, but because it uses Near Infra-Red light it has much finer resolution (<10μm) for the same imaging depth. OCT allows one to see, in-vivo, in real time and non-invasively, tissue microstructure, without exposing the subject or user to ionizing radiation. All OCT System will enable the user to:
1. Enter and manage patient and descriptive data
2. Acquire single and multiple 2-dimensional OCT images of sub-surface tissue
3. Export OCT images as compliant image files
Users of the equipment need to be computer literate healthcare professionals, and to have undergone the appropriate level of training. There are no restrictions on patient population, and no restrictions related to which part of the body to scrutinize. Data from an OCT system are presented as two-dimensional in which

the lateral and axial dimensions correspond, respectively, to the tissue's spatial dimension perpendicular (along the surface) and parallel (along depth) to the light beam. (42)
To date, OCT is a reliable tool in ophthalmology, allowing to obtain very precise corneal and retinal scans that allow to analyze in detail the layers of the cornea, macula and optic nerve, allowing the diagnosis and follow-up of numerous corneal and retinal disorders such as diabetic retinopathy and glaucoma. Recently, the use of this method is also being promoted in dermatology. New studies have investigated the possibility of using this method in other medical specialties: general surgery, gastroenterology, pneumology, urology, ginecology. (43,44,45)

3.2 OCT and oral applications

To date, dental hard (tooth) and soft (hard palate mucosa and gingiva mucosa) tissues are visualized with OCT. In fact, potential application of OCT have been described in Restorative dentistry for diagnosis of primary and recurrent caries, as well as determination of the accuracy of composite restoration; in Endodontics, to facilitate the identification of additional pulp canals; in Implantology, for intraoperative localization of anatomical sites such as inferior alveolar canal and floor of sinus maxillae, and in Periodontology, to determine the extent of alveolar bone loss in patients with periodontitis. (46)
Compared to the radiographic examination, OCT provides a higher spatial resolution and contrast for hard tissue and allows the discrimination of enamel and dentin. Moreover, OCT overcomes the drawbacks of superimposed imaging with ionizing radiation and enables the observation of short-term progression in carious dental hard tissue.(47)

Recently, OCT was experimented for detecting periimplantitis: the results of ex vivo studies are promising in indicating OCT as an helpful tool to prevent peri-implant disease. (48)
OCT is useful for evaluating the presence of subgingival calculus on the root surface and therefore may be suited as imaging technology for subgingival calculus in periodontal pockets. (49)
Concerning oral medicine, some articles focused on the usefulness of in vivo OCT for diagnosing oral soft tissues lesions, with the aim to compare the OCT results with traditional histology.
A recent review (50) said that OCT is not only an alternative method to detect oral cancer and precancerous lesions but also to for diagnosing and monitoring some pathological conditions such as chemotherapy-induced oral mucositis, autoimmune / bullous diseases.
The main perk of OCT in oral medicine would consist in providing a description of the pathological changes in soft tissues that normally would require a confirmation through biopsy, and to intercept worrisome alteration of obvious

lesions, which may otherwise be overseen or underestimated, leading to a delayed detection of malignant pattern (51).

Regarding oral cancer, OCT provides assessment of the entire epithelium and the underlying lamina propria, highlighting a disruption of the basement membrane, a thorough irregularity in the vasculature, with an overturn of the backscattering of the altered tissue lesion, appearing as an image of brighter intensity at the surface of the scan fading off with depth. (52-53)

One of the most recent advances consisted in the association of OCT technology with artificial neural networks, which might be able to dramatically improve the reliability in interpretation of images, with a far more precise pattern-finding algorithm in distinguishing benign, premalignant and malignant lesions. A very recent paper offered some encouraging results, with an estimated sensitivity and specificity of 100% and 70%, respectively, whenever such combination was carried out. (54)

Furthermore, oral manifestations of systemic diseases can trigger oral lesions in need of recurrent treatment: in this sense, OCT has been tested as well, and used to observe and evaluate the consequences of different therapies.

Duong et al (55), in a double-blind study, used OCT to analyze microscopic changes in patients with xerostomia in response to a dry mouth toothpaste versus fluoride toothpaste placebo. The authors were able to detect and measure oral epithelial response to the use of a dry mouth toothpaste in patients diagnosed with moderate to severe xerostomia.

Similarly, a study confirmed that OCT imaging was more sensitive in anticipating early mucositis compared to the clinical parameters assigned by physician. (56)

Finally, OCT has been tested as a tool for clinicians to distinguish epithelial and sub-epithelial pattern in patients affecting by oral bullous diseases, with encouraging evidence, although histological examination and immunofluorescence methods remain the gold-standard. (57)

SECTION 2

Chapter 4

4.1 Aims of the study

The aims of our project were divided in two parts:

AIM 1**:** to compare the image obtained from the OCT after usage on the oral mucosa of patients with erosive and painful OLP to the traditional histopathology slides seen in the electron microscope in order to create a common pattern between pathologist and clinical, and then evaluate images in vivo on a patient.

AIM 2: to evaluate, through the use of OCT, morphometric changes of the oral tissues of patients with erosive and painful OLP that have performed drug topical steroid therapy (Group A) and to compare them with laser-PBM (Group B).

4.2 AIM 1

4.2.1 Methods

Patients

A case-control approach was carried out, with the following selection criteria for "case group" and "control group":

Case group
First phase: selection by disease
Patients were selected among those referred to the Department of Oral Medicine, Dental School, Turin, either for a first histologic diagnosis of OLP, or as patients already diagnosed with OLP, undergoing clinical follow-up. Specifically, patients with symptomatic atrophic-erosive OLP not exposed to any topical or systemic

corticosteroid treatment in the previous 4 weeks were considered eligible for a first selection.

Second phase: selection by site

Of the patients with clinic-histologically confirmed atrophic-erosive OLP, only patients with atrophy or erosion signs of OLP within the mucosa of the cheek were asked to participate to OCT analysis. Cheek was considered the most reliable anatomic site to be scanned with OCT, since it appeared to be the easiest to be kept in hyperextension by the oral physician for 30 seconds, with minimal to no complaint from the patient. Furthermore, with its plain surface, cheek was by far the most appropriate site to maintain a constant contact with the OCT probe, rather than gingiva, where the irregularities of the underlying bone could compromise the quality of the scan, or the tongue, due to the difficulties for the tongue to be kept still for the whole time necessary to complete the scan.

Control group

Controls were selected among patients referred to our Department for excision of traumatic benign lesions of the cheek (i.e. irritational fibroma, fibrous hyperplasia). In these cases, a wider diameter of the surrounding healthy buccal mucosa was included in the surgical lozenge and detached from the original specimen, in order to be evaluated as healthy mucosa by the pathologist.

Pathologic evaluation

Pathologist was asked to describe the following parameters: keratin layer, epithelial layer, basement membrane, and lamina propria with the corresponding width of each layer for both healthy and affected mucosa.

He was asked to provide within the histological report precise measurements, expressed through a millimiter scale, of width of the following layers: keratin layer, epithelial layer and lamina propria.

For the latter, further parameters were asked, as follows: hyperparakeratosis, acanthosis, spongiosis for the epithelial layer, persistence or disappearance of the basement membrane, and characteristics of the inflammatory infiltrate within the lamina propria.

4.2.2 Results

OCT appearance: healthy mucosa at enface scan

A recurrent pattern of healthy oral mucosa of the cheek was detected in the control group (**Fig.1**): the cross-sectional OCT scan revealed a light-grayish, hyporeflective, homogeneous area, with an approximate width of 260-300 μm, corresponding to stratified squamous epithelium (EP). With no significant hyper-reflectiveness throughout EP area, and especially in its upper layers, we were able to infer the absence of a keratinized layer, as expected in the epithelium of a lining mucosa.

Beneath EP, a whitish, hyper-reflective, non-homogenous area, with an approximate width of 600 μm was detected, corresponding to the underlying lamina propria (LP).

The difference in reflectiveness and homogeneity between EP and LP might be caused by the overall homogeneity of EP, leading to a lower backscattering signal, whereas LP, a dense fibrous connective tissue with embedded blood vessels and nerves, might display a more non-homogeneous pattern, leading to a higher backscattering signal in the OCT cross-sections.

Underneath LP, where the deepest layers of connective/muscular tissue are histologically detectable, a homogenous dark area appears, suggestive of the ultimate boundary of OCT scan, preventing from further evaluation.

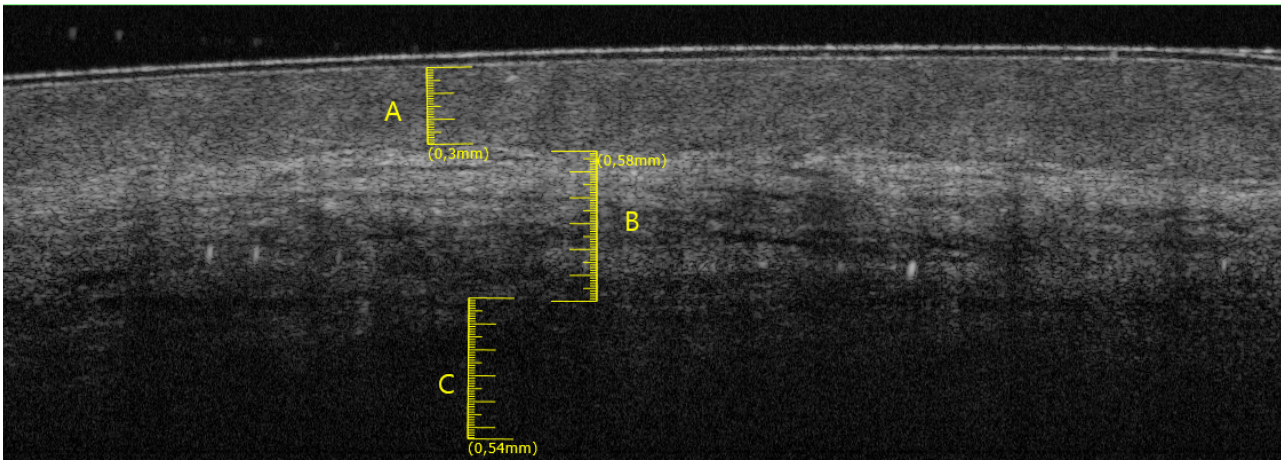


FIGURE 1. Typical pattern of healthy buccal mucosa at enface scan. **A:** stratified squamous epithelium (EP) as grayish, hyporeflective, homogeneous area; **B:** underlying lamina propria (LP) as hyper-reflective, non-homogenous area; **C:** deepest layers of connective/muscular tissue as unreadable homogenous dark area.

OCT appearance: healthy mucosa at dynamic scan

Cross-sectional dynamic scan is able to reveal the vascularization within LP, where the dense fibrous connective tissue with embedded small caliber blood vessels can be seen as a hypo-reflective red area with a mottled pattern, intertwined through serpiginous red "spikes" with the overlying epithelium. In the homogeneous dark area beneath lamina propria, in which the enface scan could not allow any further analysis, this red mottled pattern emerges, as well (**Fig.2**).

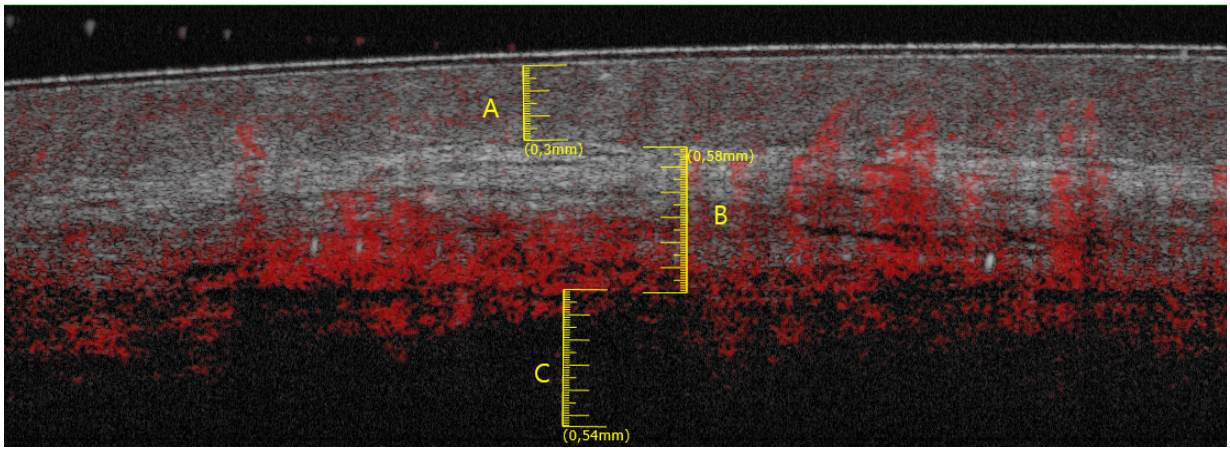


FIGURE 2. Typical pattern of healthy buccal mucosa at dynamic scan. **A:** stratified squamous epithelium (EP) displaying overlapping characteristics as in Fig.1A;
B: vascularization within LP, as a hypo-reflective red area. Notice the mottled pattern, and the serpiginous red "spikes" at the interface with overlying EP. **C:** red mottled pattern emerging partially within the homogeneous dark area beneath LP

Histological appearance of healthy mucosa

It must be pointed out that the measurements provided by the pathologist in his descriptive report are calculated in an archived, formalin-fixed specimen. Such values must then be interpreted carefully, since they have been obtained from a sample of tissue which was exposed to significant alterations in length, width and depth (ie crystallization, contraction) throughout the processing **(Fig. 3).**

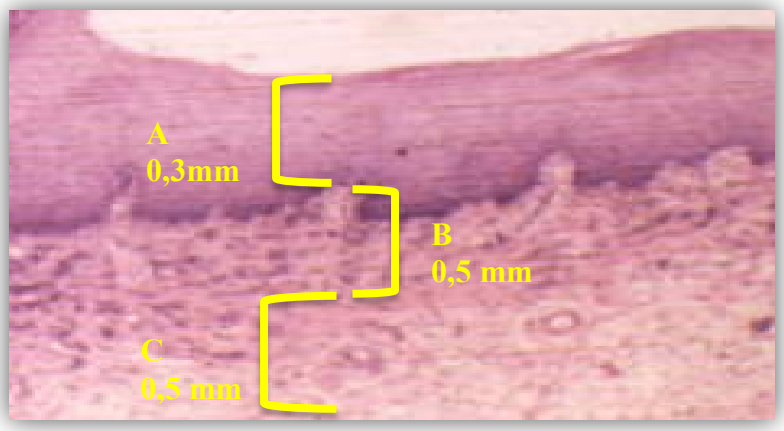


FIG 3. **A:** stratified squamous epithelium (EP) **B:** underlying lamina propria (LP) **C:** connective/muscular tissue with blood vessels corresponding to dark area. The misurements have a general agregment with OCT images.

OCT appearance: OLP mucosa at enface scan

The main and most recurring change of OLP mucosa at enface scan could be found within LP (**Fig.4**), where the distinct hyper-reflectiveness area is almost completely lost, leading to further difficulties in recognizing the transition between the overlying epithelium and the underlying connective tissue. On the other hand, the epithelium itself can present less width, and an unexpected higher propensity for hyper-reflectiveness, indicative of either hyperkeratosis or hyperparakeratosis.

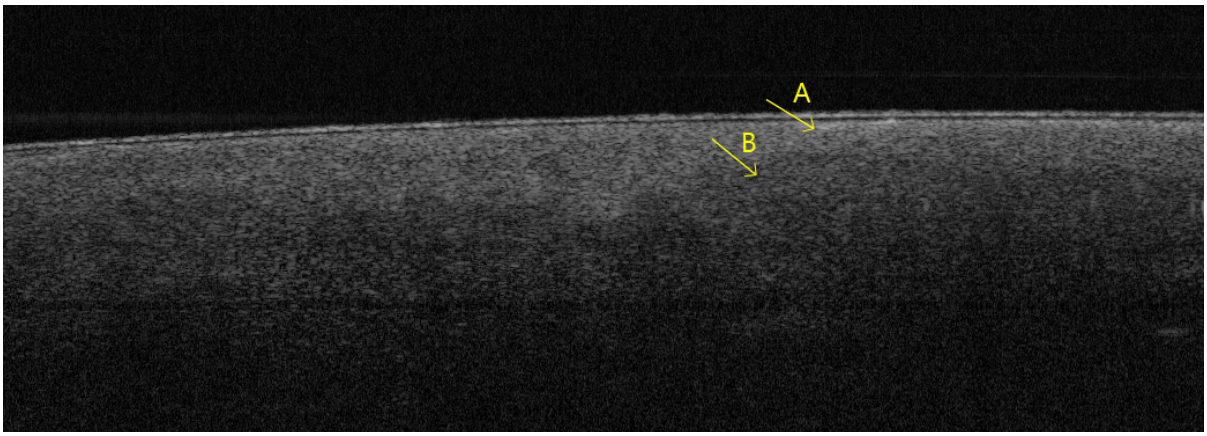


FIGURE 4. Typical pattern of buccal mucosa affected by OLP at enface scan.
A: EP with less width, and higher hyper-reflectiveness, indicative of either hyperkeratosis or hyperparakeratosis; **B:** LP with almost complete lack of distinct hyper-reflectiveness and of a clear transition between EP and LP.

OCT appearance: OLP mucosa at dynamic scan

The main and most recurring change of OLP mucosa at the dynamic scan is the scattered red dots emerging throughout the homogenous gray hyporeflectiveness of EP. Such condition might be attributed to the concurrent intra and inter-cellular oedema, as expected in cases of acanthosis and spongiosis, typical ultrastructural manifestations of OLP. On the other hand, LP reveals, together with the loss of integrity and hyper-reflectiveness of the lamina propria, an increased, denser red pattern of vascularization, indicative of a higher blood inflow within a chronically-inflamed mucosa (**Fig.5**).

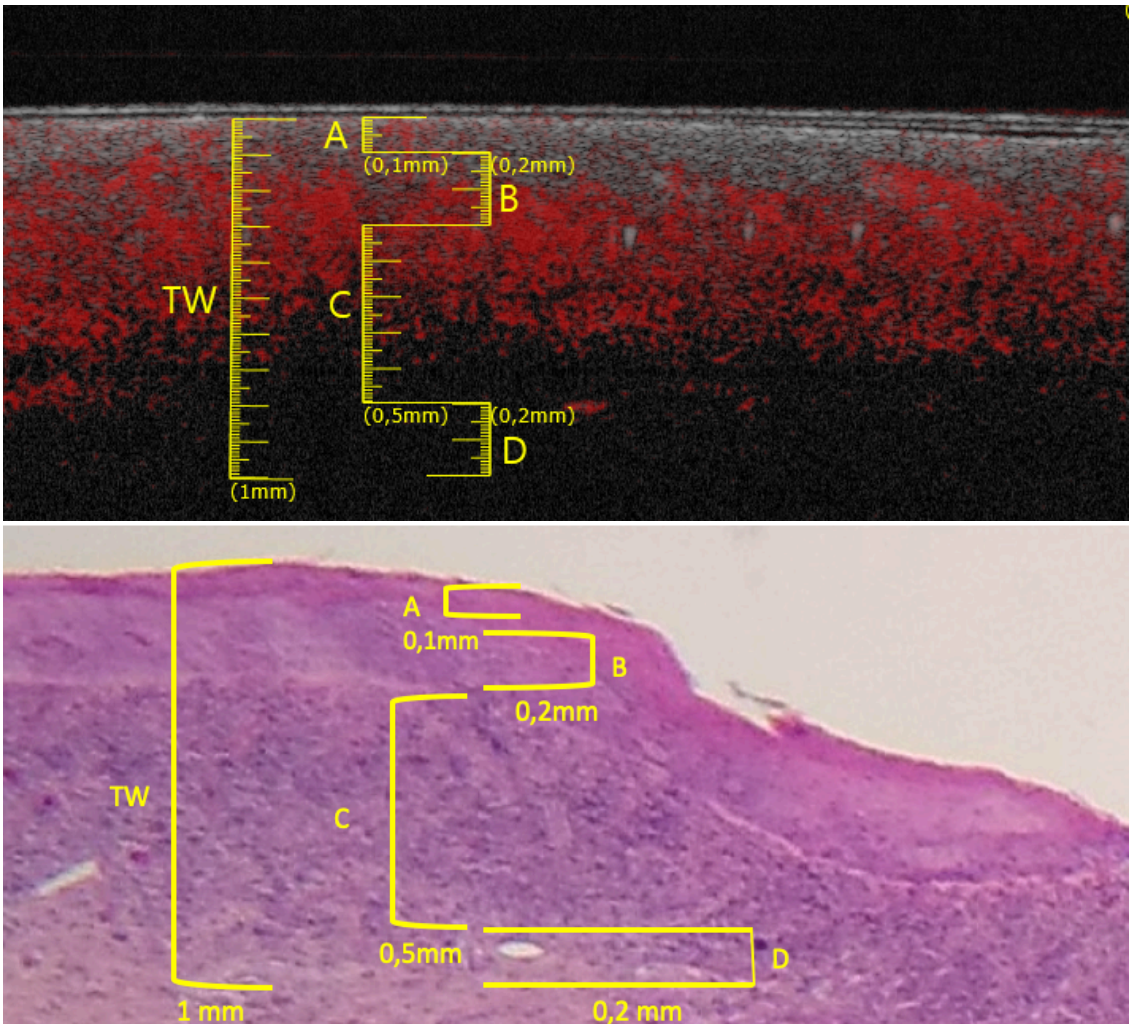


FIGURE 5. Typical pattern of buccal mucosa affected by OLP at dynamic scan and comparison with histological specimen. **A:** scattered red dots in the first 100 μm of EP, corresponding to the pathologic finding of paracheratosis and granulocyte exocytosis. **B:** scattered red dots in the underlying 200 μm of EP, corresponding to the pathologic finding of lymphocyte and granulocyte exocytosis; **C:** enriched inflammatory infiltrate within the 500 μm LP. Notice the increased, denser red pattern of vascularization when compared to 2B, indicative of a higher blood inflow typical of a chronically-inflamed mucosa **D:** remaining layers of the deepest fibrous stroma, as a dark, unreadable area. **TW:** total width of the sample (1 mm).

Chapter 5

5.1 AIM 2

Aim 2 was to evaluate, through the use of OCT, morphometric changes of the oral tissues of patients with erosive and painful OLP undergoing drug topical steroid therapy (Group A) compared to PBM therapy (Group B). Each lesion undergoing such treatment would be evaluated with OCT right before and after treatment, as well as at six months after the conclusion of both eight-weeks protocols.

5.2 METHODS

Patients

Two groups (Group A and B) of 20 patients each were forged, selected from a random original sample of 100 patients affected by erosive and painful OLP, referred to the Department of Oral Medicine, CIR Dental School, Turin, either for first clinic-histological diagnosis of OLP, or for a follow-up visit, unexposed to any topical or systemic corticosteroid treatment in the previous 8 weeks. Each patient was informed of our protocol and signed an informed consent, whenever keen to participate.

Therapy

Group A would undergo a eight weeks "gold-standard" treatment with two daily application of clobetasol dipropionate 0.05% in an aqueous gel of 4% hydroxyethyl cellulose (100 g) in equal parts (50:50).
Group B would be expose to eight PBM sessions – once a week for eight weeks - with "Raffaello diode laser" 980/645 nm. In accordance with the manufacturer's instructions, the device was used with the following parameters: output power = 300 mW, power density = 0,8 W/cm^2, fluence = 8 J/cm^2, collimated probe of 0.6 cm in diameter and spot size of 0.28 cm^2, kept perpendicularly at 2 mm from the area of irradiation. A "spot" technique with a slight overlapping would be carried out in each site, in order to distribute energy evenly on the mucosal lesions and the perilesional tissues up to 0.5 cm for 10 seconds.

OCT measurements

OCT scans were performed before initiating the treatment protocol, and repeated at the end of the 8-weeks treatment protocols for both groups . Photographs of the lesions were acquired before and at the end of treatment. The changes of width within the stratified epithelium (EP) and the lamina propria were standardized as follows:

1. the 60th frame of either enface or dynamic scan was taken as "gold standard" for the analysis, being at the exact center of the 120 scans provided by the OCT machine used in the present work, coinciding with the very center of the lesion, and being as much refined as possible from artifacts either caused by patients' sudden movements or by clinician's excessive pressure, which can sometimes be experienced at the beginning or at the end of the scanning process and can be observed in the first or last frames of the scan.

2. EP width of the 60th frame was regularly measured through the dynamic scan as follows: the light-grayish, hyporeflective, homogeneous area intertwined between the plastic wrapping and the level at which the peak of the red spikes occurred most frequently, thus indicating the transition from the epithelium to the underlying vascularized tissue of LP **(Fig.6)**

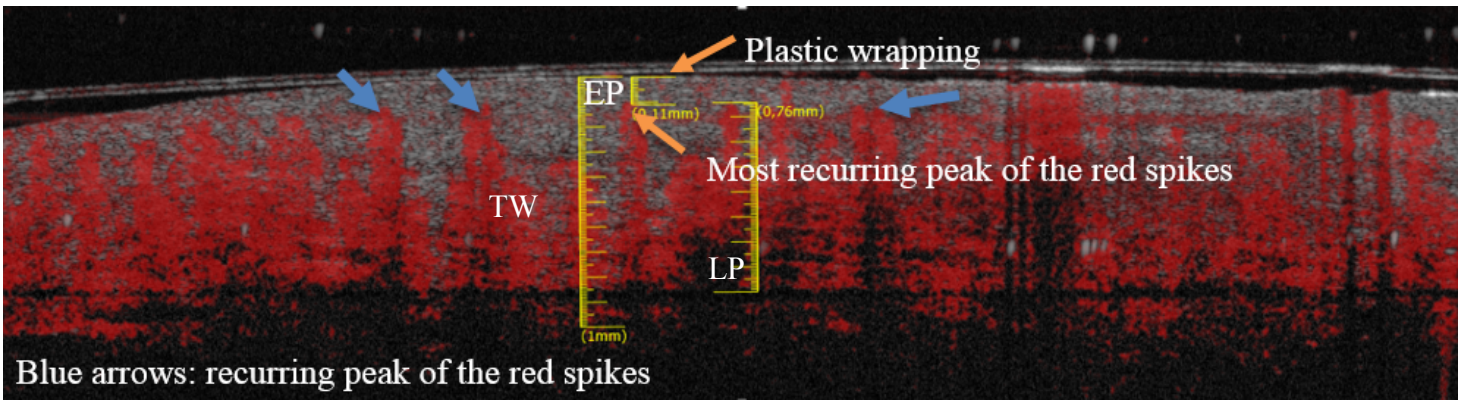


Fig.6. OCT (dynamic scan): standardized method of EP measurement.

3. LP width of the 60th frame was regularly measured through the dynamic scan as follows: the hypo-reflective red area intertwined between the most recurring peak of the red spikes and the most recurring position of the base of the red spikes, thus indicating the transition between LP and the homogenous, unreadable dark area **(Fig.7).**

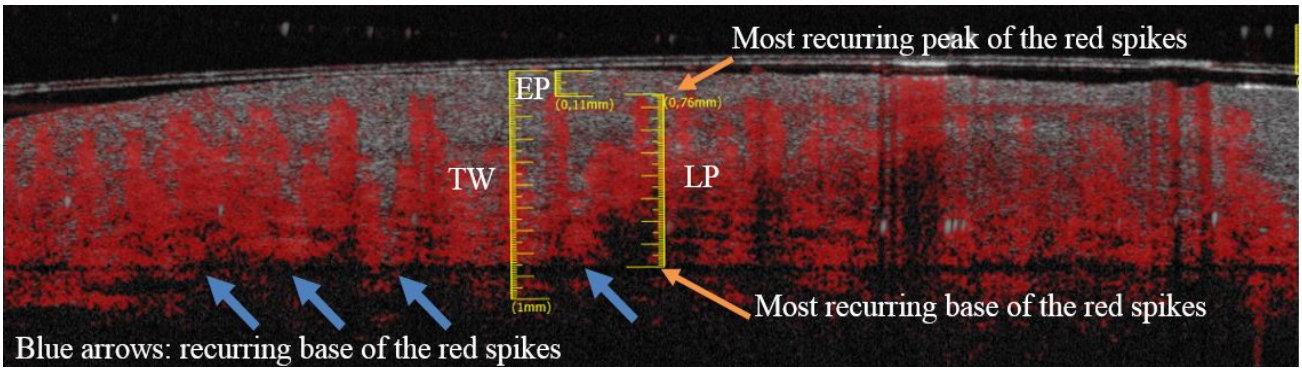


Fig.7. OCT (dynamic scan): standardized method of LP measurement.

Statistical analysis

Paired t-student test was conducted to evaluate the variations of EP and LP width within Group A and Group B, both at the end of treatment, and six months after the end of treatment. On the other hand, unpaired t-student test was performed to evaluate differences in fluctuations of EP (Δ-EP) and of LP (Δ-LP) between Group A and B. Statistical analysis was performed using SAS ver 9.3, and 2-tails p-value less than 0.01 was considered statistically significant.

5.3 RESULTS

Group A: clobetasol propionate

Group A revealed a significant variation of both EP and LP between the beginning and the end of the 8-weeks protocol with clobetasol propionate: Specifically, EP experienced an overall increase after the treatment (**Table 1)**, from a mean width of 0.14 (±0.02) mm to 0.19 (±0.03) (Table 2). Paired t test revealed a two-tailed P value <0.0001 (95% CI: −0.0651- −0.0379), suggesting a statistically significant increase of EP width. **(Table 2).**

Table 1. Measurements of EP width before and after clobetasol treatment of Group A patients.

Patients	EP width before clobetasol treatment (mm)	EP width after clobetasol treatment (mm)
1	0.11	0.19
2	0.16	0.22
3	0.15	0.2
4	0.12	0.18
5	0.13	0.18
6	0.19	0.2
7	0.16	0.22
8	0.14	0.2
9	0.16	0.21
10	0.17	0.21
11	0.12	0.12
12	0.11	0.19
13	0.13	0.17
14	0.15	0.15
15	0.18	0.22

16	0.17	0.26
17	0.13	0.25
18	0.11	0.17
19	0.15	0.18
20	0.15	0.2

Table 2. Mean, SD, SEM of EP width variations in Group A - before and after clobetasol treatment.

Statistical parameters	**EP width before clobetasol treatment (mm)**	**EP width after clobetasol treatment (mm)**
Sample size	20	20
Mean	0.1445	0.1960
SD (standard deviation)	0.0239	0.0319
SEM (standard error of mean)	0.0054	0.0071

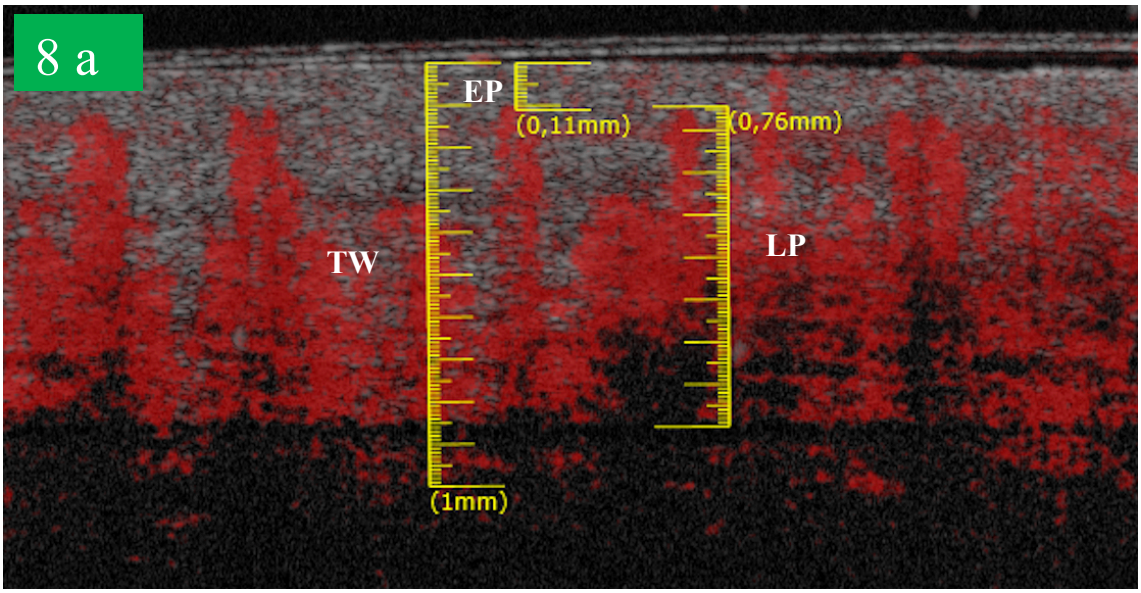
On the other hand, LP experienced an overall decrease after clobetasol treatment **(Table 3)**, shifting from a mean width of 0.68 (± 0.04) mm to 0.64 (± 0.04) mm. Paired t test revealed a two-tailed P value <0.001 (95% CI: 0.0183-0.0537), suggesting a statistically significant decrease of LP width. **(Table 4).** Figure 1 shows the variation of EP and LP in patient 1 before and after treatment.

Table 3. Measurements of LP width before and after clobetasol treatment of Group A patients.

Patients	LP width before clobetasol treatment (mm)	LP width after clobetasol treatment (mm)
1	0.76	0.61
2	0.61	0.61
3	0.68	0.62
4	0.66	0.65
5	0.65	0.63
6	0.67	0.60
7	0.64	0.59
8	0.68	0.65
9	0.64	0.60
10	0.69	0.67
11	0.62	0.60
12	0.63	0.63
13	0.65	0.65
14	0.70	0.61
15	0.71	0.66
16	0.77	0.72
17	0.72	0.69
18	0.67	0.67
19	0.70	0.71
20	0.72	0.68

Table 4: Mean, SD, SEM of LP width variations in Group A before and after clobetasol treatment.

Statistical parameters	**LP width before clobetasol treatment (mm)**	**LP width after clobetasol treatment (mm)**
Sample size	20	20
Mean	0.6785	0.6425
SD (standard deviation)	0.0436	0.0386
SEM (standard error of mean)	0.0097	0.0086



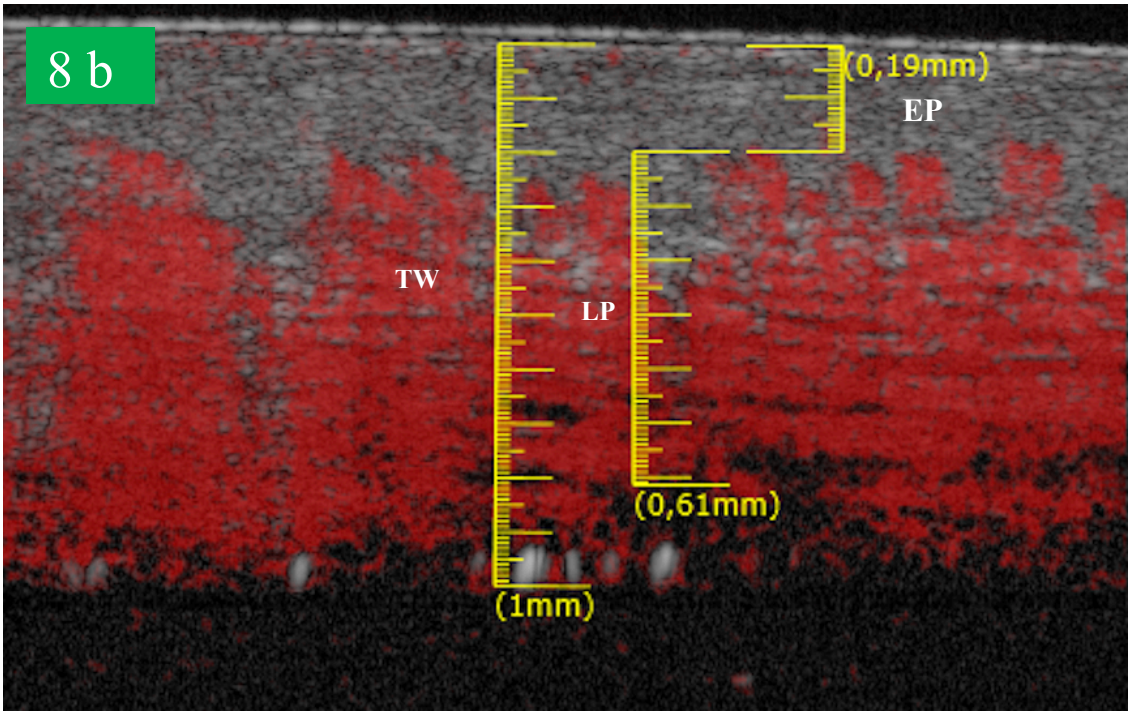


Fig.8. OCT (dynamic scan): Patient 1 of Group A: **8a**: EP and LP width before treatment; **8b**: EP and LP width after treatment. Notice the increase of EP (from 0.11 to 0.19 mm), and the corresponding decrease of LP (from 0.61 to 0.76 mm)

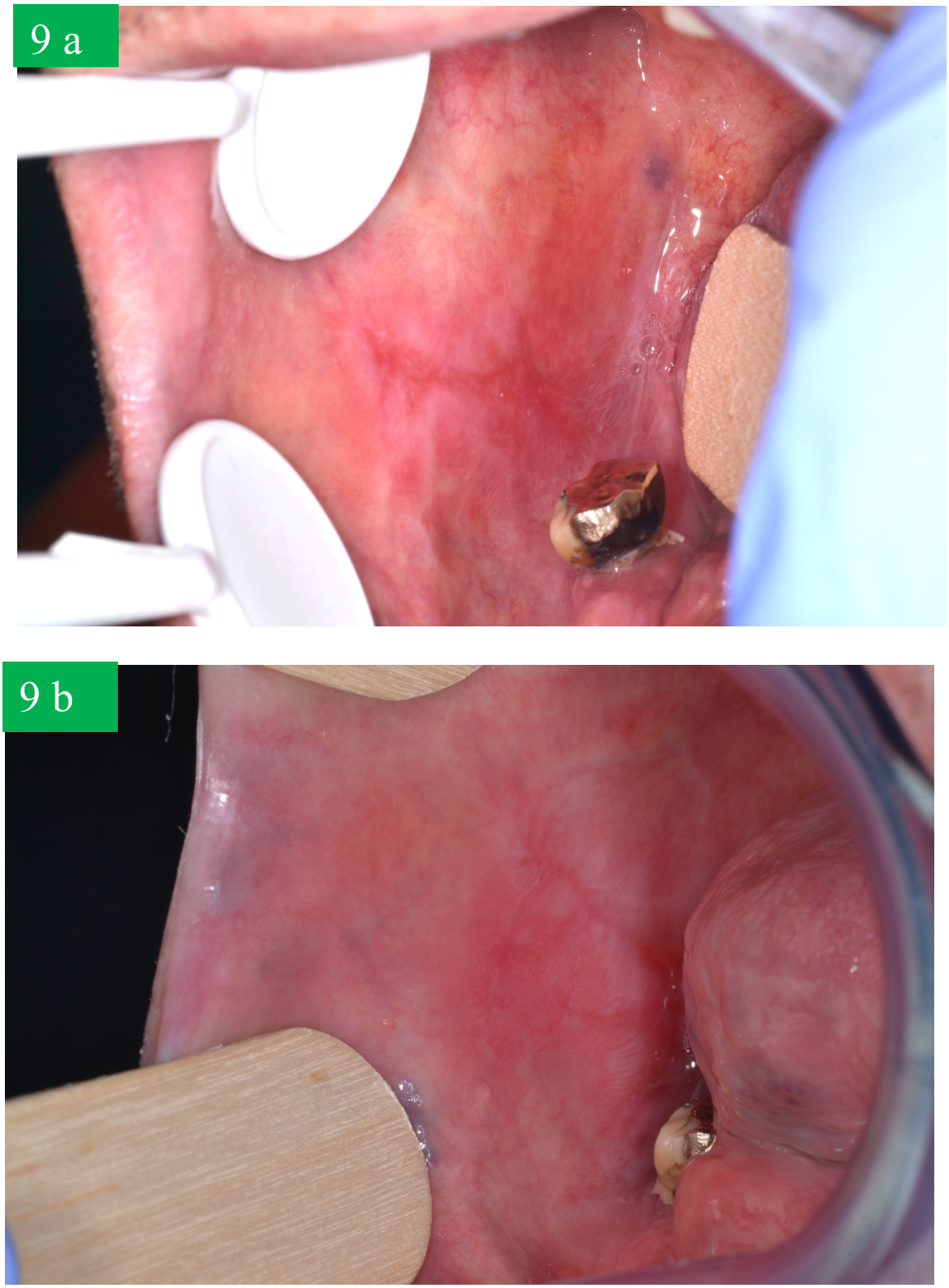


Fig. 9: Clinical appearance of patient 1 before (9a) and after (9b) topical steroid treatment.

Clinically, only 12 of 20 (60%) were able to undergo no treatment for six months, with the remaining eight (40%) patients forced to recur to clobetasol treatment

(four patients: 1 month later, three patients: between 2 and 4 months; one patient: 5 months later): thus, the measurements were acquired and registered only in 12 cases. Bearing this 40% dropout rate in mind, six months after the end of treatment, the aforementioned variations were not maintained, with EP and LP width showing an almost overlapping pattern to pre-therapy measurements. Specifically, EP values after six-months displayed a mean value of 0.145 (± 0.02) mm, very close to mean width of 0.143 (±0.02) mm registered by these 12 patients before therapy **(Table 5)**. Paired t test revealed a two-tailed P value = 0.74 (95% CI: −0.0124 - 0.0091), suggesting no statistically significant differences of EP width **(Table 6)**. Figure 2 shows the variation of EP and LP width between the beginning and the end of the six months protocol.

Table 5. Measurements of EP width before clobetasol treatment and six months after end of treatment

Patients	EP width before clobetasol treatment (mm)	EP six months after end of clobetasol treatment (mm)
1	0.11	0.13
2	0.16	0.15
3	0.15	0.14
4	0.12	0.15
5	0.13	0.12
6	0.19	0.19
7	0.16	0.17
8	0.14	0.14
9	0.16	0.15
10	0.17	0.14
11	0.12	0.14
12	0.11	0.13

Table 6. Mean, SD, SEM of EP width variations in Group A before clobetasol treatment and six months after end of treatment

Statistical parameters	EP width before clobetasol treatment (mm)	EP width six months after end of clobetasol treatment (mm)
Sample size	12	12
Mean	0.1433	0.1450
SD (standard deviation)	0.0257	0.0198
SEM (standard error of mean)	0.0074	0.0057

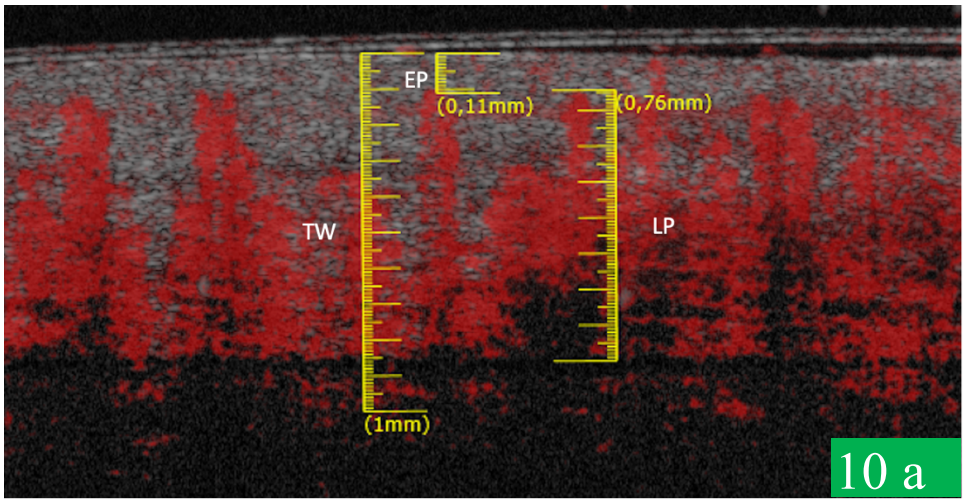
Similarly, LP measurements after six months displayed an overlapping pattern to those registered in these 12 patients before treatment **(Table 7)**, with a mean value of 0.6608 (± 0.04) mm, being very close to a mean pre-treatment width of 0.674 (± 0.04) mm. Paired t test revealed a two-tailed P value = 0.23 (95% CI: −0.0365 - 0.0099), suggesting no statistically significant differences. **(Table 8**).

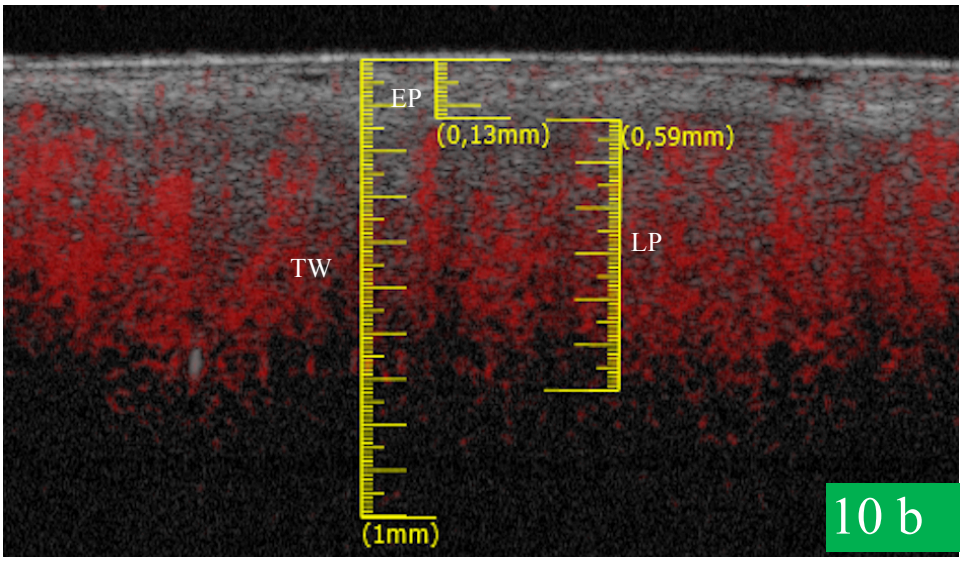
Table 7. Measurements of LP width before clobetasol treatment and six months after end of treatment

Patients	LP width before clobetasol treatment (mm)	LP width six months after end of clobetasol treatment (mm)
1	0.76	0.59
2	0.61	0.65
3	0.68	0.70
4	0.66	0.72
5	0.65	0.69
6	0.67	0.73
7	0.64	0.62
8	0.68	0.66
9	0.64	0.65
10	0.69	0.70
11	0.62	0.71
12	0.63	0.67

Table 8. Mean, SD, SEM of LP width variations in Group A before clobetasol treatment and six months after the end of clobetasol treatment.

Statistical parameters	**LP width before clobetasol treatment (mm)**	**LP width six months after clobetasol treatment (mm)**
Sample size	12	12
Mean	0.6608	0.6742
SD (standard deviation)	0.0401	0.0421
SEM (standard error of mean)	0.0116	0.0122





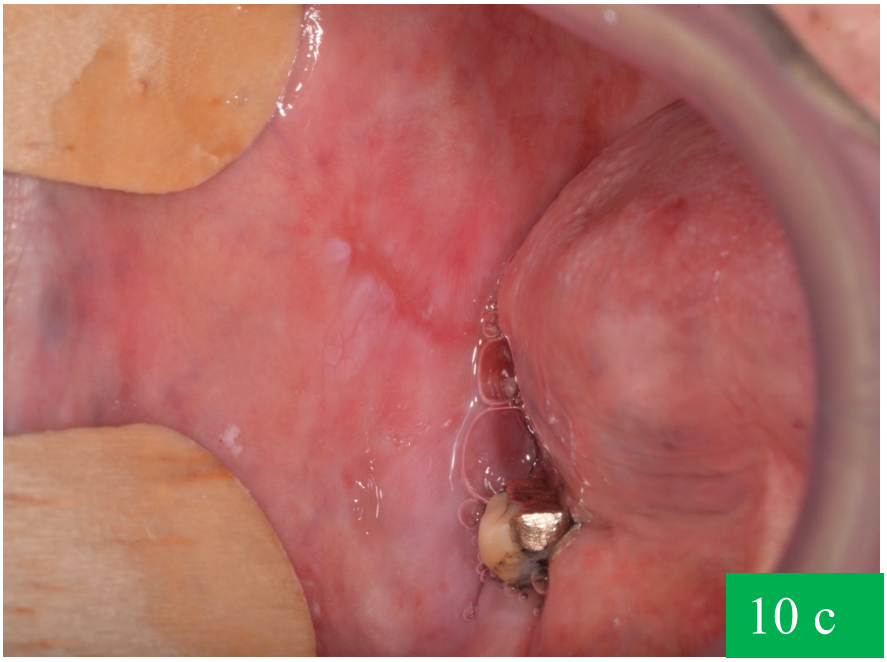


Fig.10 OCT (dynamic scan): Patient 1 of Group A: **10a**: EP and LP width before treatment; **10b**: EP and LP six months after end of treatment. Notice the increase of EP (from 0.11 to 0.13 mm) and the corresponding decrease of LP (from 0.76 to 0.59 mm), corresponding to a partial clinical improvement (**10c**) when compared to figure 9a.

Group B: laser-PBM

As in Group A, Group B measurement experienced a significant variation of both EP and LP between the beginning and the end of the 8-weeks laser-PBM protocol: Specifically, EP experienced an overall increase after the treatment **(Table 9)**, from a mean width of 0.16 (±0.02) mm to 0.18 (± 0.02). Paired t test revealed a two-tailed P value = 0.0003 (95% CI: −0.33 - 0.01), suggesting a statistically significant increase of EP width. **(Table 10)**.

Table 9. Measurements of EP width before and after laser-PBM treatment of Group B patients.

Patients	EP width before laser-PBM treatment (mm)	EP width after laser-PBM treatment (mm)
1	0.19	0.2
2	0.15	0.13
3	0.15	0.21
4	0.18	0.21
5	0.17	0.19
6	0.13	0.15
7	0.13	0.17
8	0.16	0.22
9	0.18	0.2
10	0.17	0.2
11	0.12	0.17
12	0.2	0.19
13	0.2	0.19
14	0.11	0.14
15	0.15	0.18
16	0.15	0.16

17	0.16	0.16
18	0.12	0.17
19	0.18	0.19
20	0.19	0.21

Table 10. Mean, SD, SEM of EP width variations in Group B before and after laser-PBM treatment.

Statistical parameters	EP width before laser-PBM treatment (mm)	EP width after laser-PBM treatment (mm)
Sample size	20	20
Mean	0.1595	0.1820
SD (standard deviation)	0.0274	0.0250
SEM (standard error of mean)	0.0061	0.0056

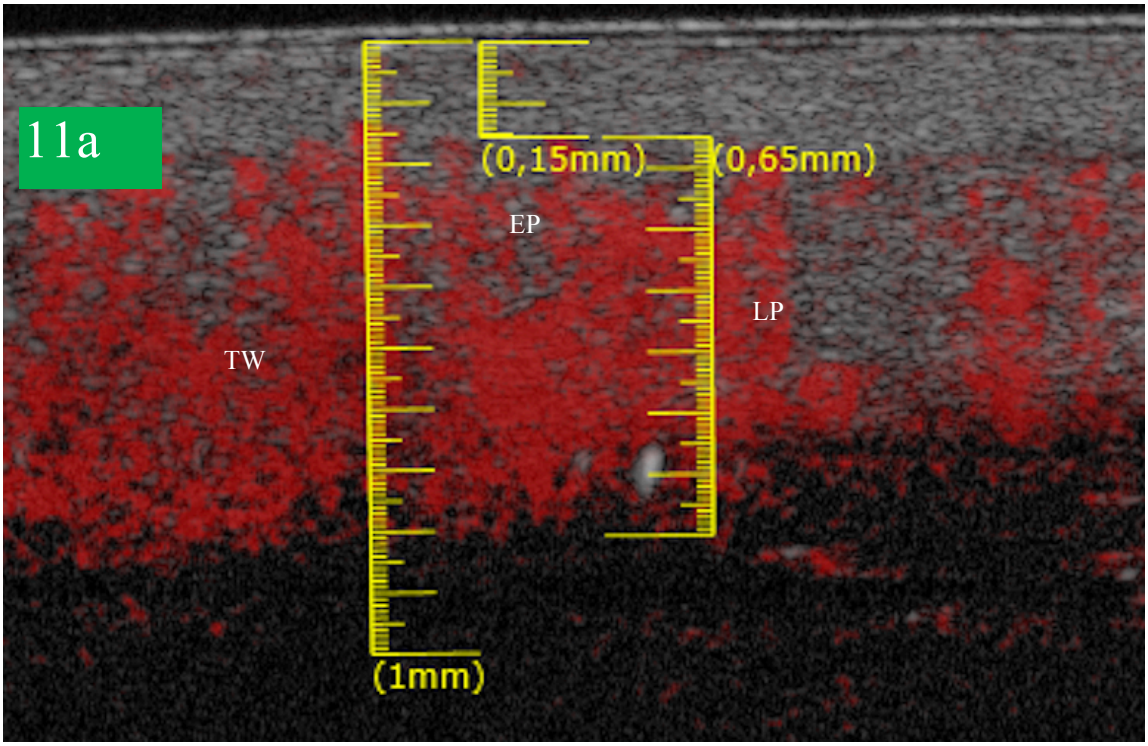
On the other hand, LP experienced a decrease after laser-PBM treatment (**Table 11)**, diminishing from a mean width of 0.69 (± 0.04) mm to 0.66 (± 0.04) mm. Paired t test revealed a two-tailed P value = 0.0068 (95% CI: 0.0104-0.0566), suggesting a statistically significant decrease of LP width. (**Table 12**).

Table 11. Measurements of LP width before and after laser-PBM treatment of Group B patients

Patients	LP width before laser-PBM treatment (mm)	LP width after laser-PBM treatment (mm)
1	0.69	0.65
2	0.65	0.72
3	0.65	0.73
4	0.67	0.59
5	0.72	0.60
6	0.75	0.69
7	0.76	0.66
8	0.63	0.63
9	0.64	0.60
10	0.68	0.65
11	0.74	0.69
12	0.71	0.68
13	0.66	0.63
14	0.76	0.73
15	0.74	0.70
16	0.70	0.64
17	0.69	0.60
18	0.62	0.62
19	0.68	0.68
20	0.68	0.66

Table 12. Mean, SD, SEM of LP width variations in Group B before and after laser-PBM treatment

Statistical parameters	LP width before laser-PBM treatment (mm)	LP width after laser-PBM treatment (mm)
Sample size	20	20
Mean	0.6910	0.6575
SD (standard deviation)	0.0433	0.0442
SEM (standard error of mean)	0.0097	0.0099



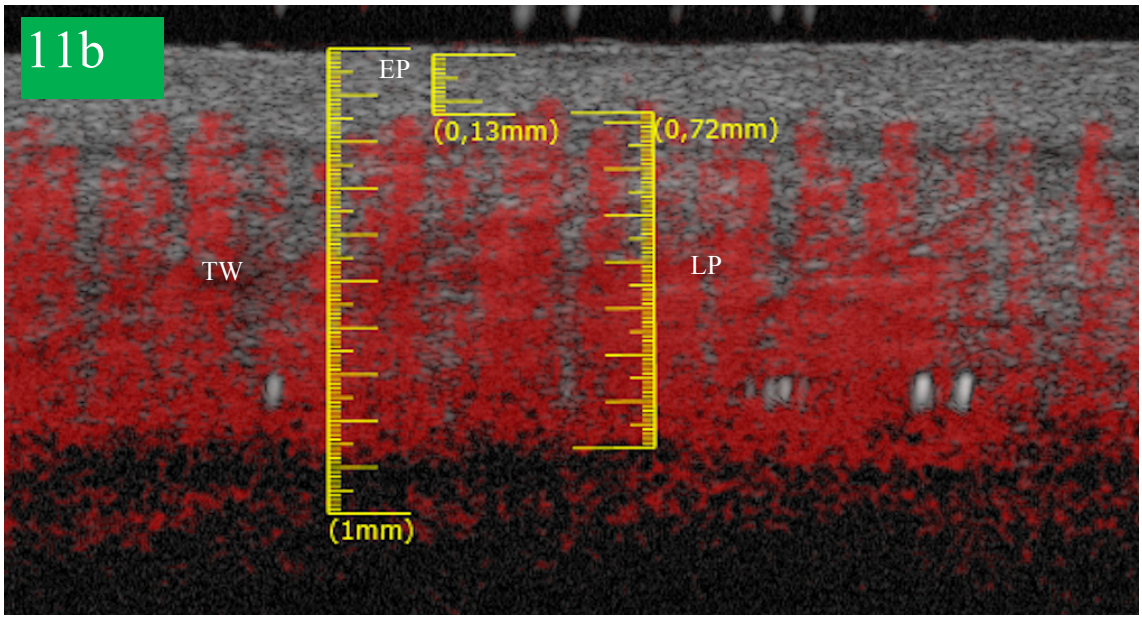
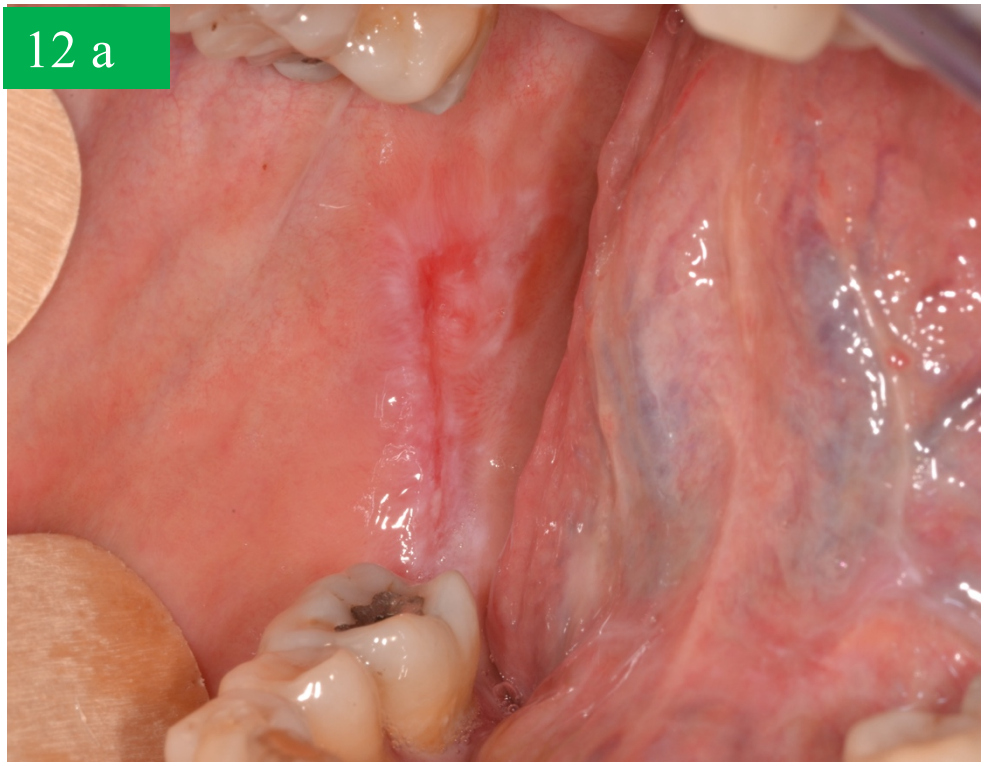


Fig. 11. OCT (dynamic scan): Patient 2 of Group B. **11a**: EP and LP width before treatment; **11b**: EP and LP width after treatment. Notice the decrease of EP (from 0.15 to 0.13 mm), and the corresponding increase of LP (from 0.65 to 0.72 mm).



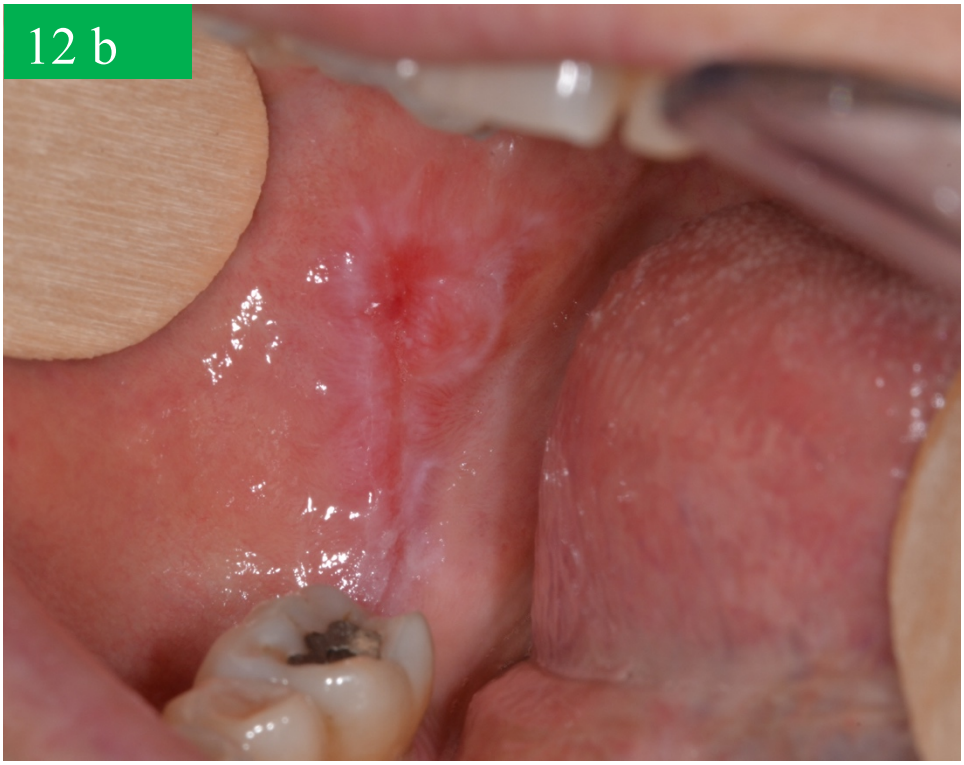


Fig. 12 : clinical appearance of patient 2 before (12a) and after (12b) PBM treatment.

Clinically, only 13 of 20 (65%) were able to undergo no treatment for six months, with the remaining 7 patients forced to recur to treatment during the last phase of the six-months protocol, either as a second cycle of laser-PBM (4 patients), or as clobetasol treatment (1 patients), or as systemic treatment in the form of prednisone tablets (2 patients). Thus, the measurements were acquired and registered only in 13 cases.

Bearing this 35% dropout-rate in mind, six months after the end of laser-PBM treatment, the aforementioned variations were not maintained, with EP and LP width showing an almost overlapping pattern to pre-therapy measurements (**Table 13**). Specifically, EP values after six-months displayed a mean value of 0.157 (± 0.03) mm, very close to mean width of 0.16 (± 0.03) mm registered before therapy. Paired t test revealed a two-tailed P value = 0.7295 (95% CI: −0.0119-0.0165), suggesting no statistically significant differences of EP width. (**Table 14**).

Table 13. Measurements of EP width before laser-PBM treatment and six months after end of laser-PBM treatment

EP width before laser-PBM treatment (mm)	EP width six months after laser-PBM treatment (mm)
0.16	0.22
0.18	0.2
0.17	0.2
0.12	0.17
0.2	0.19
0.2	0.19
0.11	0.14
0.15	0.18
0.15	0.16
0.15	0.13
0.12	0.17

0.18	0.19
0.19	0.21

Table 14. Mean, SD, SEM of EP width variations in Group B before therapy and six months after the end of laser-PBM treatment

Statistical parameters	**EP width before laser-PBM treatment (mm)**	**EP width six months after laser-PBM treatment (mm)**
Sample size	13	13
Mean	0.1600	1577
SD (standard deviation)	0.0303	0.265
SEM (standard error of mean)	0.0084	0.0074

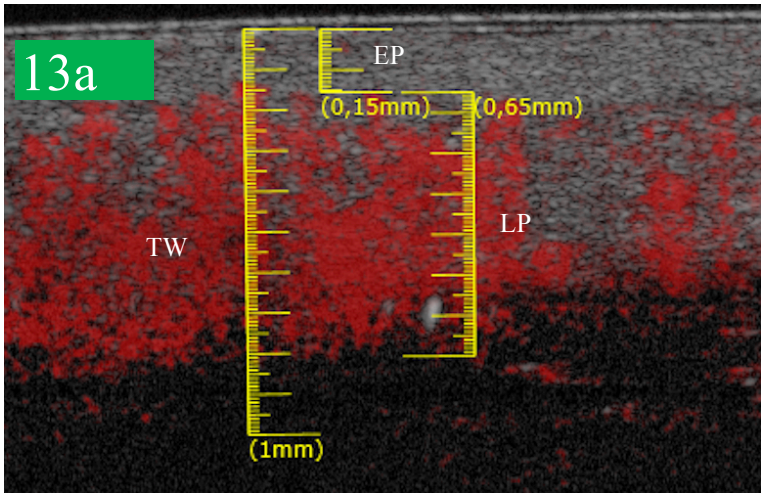
Similarly, LP measurements after six months displayed an overlapping pattern to that registered before treatment (**Table 15**), with a mean value of 0.686 (± 0.04) mm, being very close to a mean pre-treatment width of 0.695 (±0.04) mm. Paired t test revealed a two-tailed P value = 0.35 (95% CI: −0.0274 – 0.0105), suggesting no statistically significant differences. **(Table 16**).

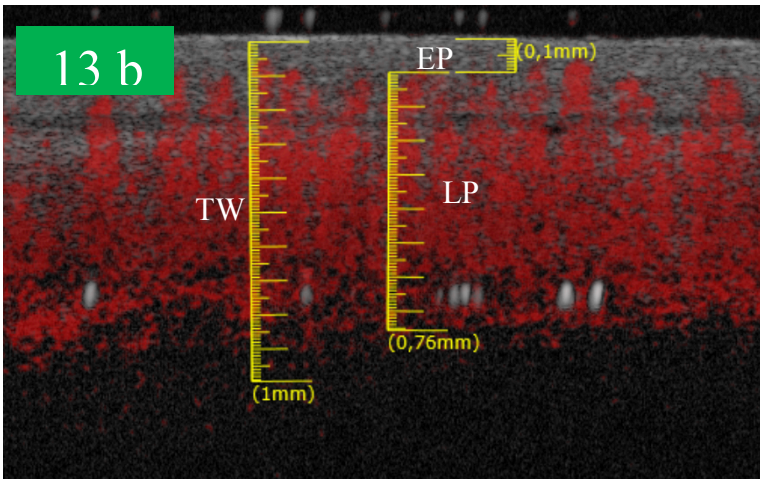
Table 15. Measurements of LP width before laser-PBM treatment and six months after end of treatment

LP width before treatment (mm)	LP width six months after treatment (mm)
0.63	0.65
0.64	0.67
0.68	0.70
0.74	0.71
0.71	0.72
0.66	0.69
0.76	0.75
0.74	0.72
0.70	0.75
0.69	0.67
0.62	0.66
0.68	0.63
0.68	0.72

Table 16. Mean, SD, SEM of LP width variations in Group B before therapy and six months after the end of treatment

Statistical parameters	LP width before treatment (mm)	LP width six months after treatment (mm)
Sample size	13	13
Mean	0.6869	0.6954
SD (standard deviation)	0.0433	0.0376
SEM (standard error of mean)	0.0120	0.0104





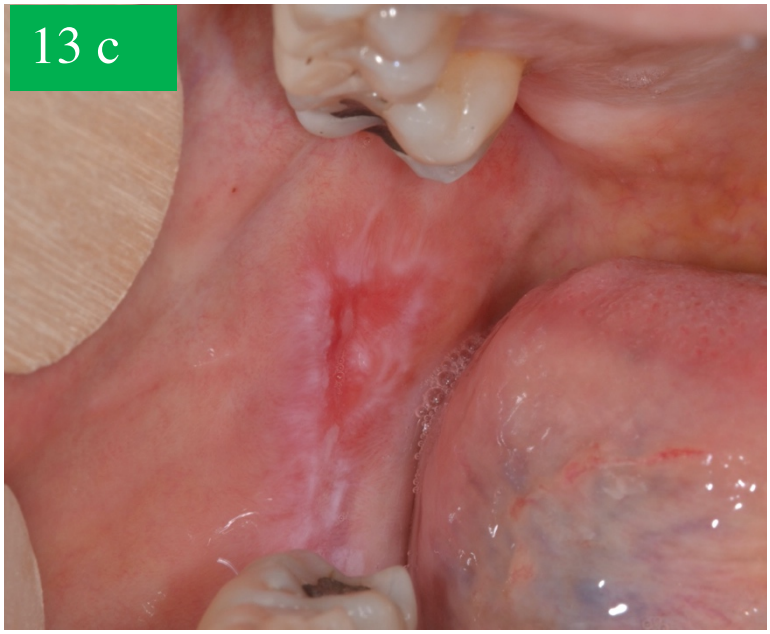


Fig.13. OCT (dynamic scan): Patient 2 of Group b: **13a**: EP and LP width before treatment; **13b**: EP and LP six months after end of treatment. Notice the further decrease of EP (from 0.15 to 0.1 mm), and the corresponding increase of LP (from 0.65 to 0.72 mm), corresponding to a partial clinical worsening (**13c**) when compared to figure 12a.

Group A VS group B at the end of 8-weeks treatment

A comparison was conducted between Group A and Group B, with the aim to assess if there were any significant differences concerning the fluctuations of EP and LP width after the 8-weeks protocols.

Therefore, the fluctuations of EP (**Δ-EP**) and LP (**Δ-LP**) width before and after treatment were calculated for both Group A (**Table 17**, **Table 20**) and Group B (**Table 18**, **Table 21**).

Secondly, the Δ-EP and Δ-LP obtained were compared through an unpaired t-Test (**Table 19**, **Table 22**).

Table 17. Δ-EP in Group A (clobetasol).

Patients	EP width before clobetasol treatment (mm)	EP width after clobetasol treatment (mm)	Δ-EP (EP after – EP before clobetasol treatment) (mm)
1	0.11	0.19	0.08
2	0.16	0.22	0.06
3	0.15	0.2	0.05
4	0.12	0.18	0.06
5	0.13	0.18	0.05
6	0.19	0.2	0.01
7	0.16	0.22	0.06
8	0.14	0.2	0.08
9	0.16	0.21	0.05
10	0.17	0.21	0.04
11	0.12	0.12	0.00
12	0.11	0.19	0.08
13	0.13	0.17	0.04
14	0.15	0.15	0.00
15	0.18	0.22	0.04
16	0.17	0.26	0.09
17	0.13	0.25	0.12
18	0.11	0.17	0.06
19	0.15	0.18	0.03
20	0.15	0.2	0.05

Table 18. Δ-EP in Group B (laser-PBM).

Patients	EP width before laser-PBM treatment (mm)	EP width after laser-PBM treatment (mm)	Δ-EP (EP after – EP before laser-PBM treatment) (mm)
1	0.19	0.2	0.01
2	0.15	0.13	−0.02
3	0.15	0.21	0.06
4	0.18	0.21	0.03
5	0.17	0.19	0.02
6	0.13	0.15	0.02
7	0.13	0.17	0.04
8	0.16	0.22	0.06
9	0.18	0.2	0.02
10	0.17	0.2	0.05
11	0.12	0.17	0.05
12	0.2	0.19	−0.01
13	0.2	0.19	−0.01
14	0.11	0.14	0.03
15	0.15	0.18	0.03
16	0.15	0.16	0.01
17	0.16	0.16	0.00
18	0.12	0.17	0.05
19	0.18	0.19	0.01
20	0.19	0.21	0.02

Group A experienced a mean Δ-EP of 0.05 (±0.03) mm, whereas Group B experienced a mean Δ-EP of 0.02 (± 0.02) mm. (**Table 19**). Unpaired t-test revealed a two-tailed P value P = 0.0015 (95% CI: 0.0119-0.0.0461), suggesting a statistically significant difference between the two groups.

Table 19. Mean, SD, SEM of Δ-EP IN Group A and Group B

Statistical parameters	**Δ-EP of Group A (clobetasol) (mm)**	**Δ-EP of Group B (laser-PBM) (mm)**
Sample size	20	20
Mean	0.0525	0.0235
SD (standard deviation)	0.0297	0.0235
SEM (standard error of mean)	0.0066	0.0052

Table 20. Δ-LP in Group A (clobetasol).

Patients	LP width before clobetasol treatment (mm)	LP width after clobetasol treatment (mm)	Δ-LP (LP after – LP before clobetasol treatment) (mm)
1	0.76	0.61	–0.15
2	0.61	0.61	0.00
3	0.68	0.62	–0.06
4	0.66	0.65	–0.01
5	0.65	0.63	–0.02
6	0.67	0.60	–0.07
7	0.64	0.59	–0.05
8	0.68	0.65	0.03
9	0.64	0.60	–0.04
10	0.69	0.67	–0.02
11	0.62	0.60	–0.02
12	0.63	0.63	0.00
13	0.65	0.65	0.00
14	0.70	0.61	–0.09
15	0.71	0.66	0.05
16	0.77	0.72	–0.05
17	0.72	0.69	-0.03
18	0.67	0.67	0.00
19	0.70	0.71	0.01
20	0.72	0.68	–0.04

Table 21. Δ-LP in Group B (laser-PBM).

Patients	LP width before laser-PBM treatment (mm)	LP width after laser-PBM treatment (mm)	Δ-LP (LP after – LP before laser-PBM treatment) (mm)
1	0.69	0.65	–0.04
2	0.65	0.72	0.07
3	0.65	0.73	0.08
4	0.67	0.59	–0.08
5	0.72	0.60	–0.12
6	0.75	0.69	–0.06
7	0.76	0.66	–0.10
8	0.63	0.63	0.00
9	0.64	0.60	–0.04
10	0.68	0.65	–0.03
11	0.74	0.69	–0.05
12	0.71	0.68	0.03
13	0.66	0.63	–0.03
14	0.76	0.73	–0.03
15	0.74	0.70	–0.04
16	0.70	0.64	–0.06
17	0.69	0.60	–0.09
18	0.62	0.62	0.00
19	0.68	0.68	0.00
20	0.68	0.66	–0.02

Group A experienced a mean Δ-LP of –0.028 (± 0.04) mm, whereas Group B experienced a mean Δ-LP of –0.030 (± 0.05) mm. (**Table 22**). Unpaired t-test revealed a two-tailed P value P = 0.87 (95% CI: –0.0282 - 0.0332), suggesting no statistically significant difference between the two groups.

Table 22. Mean, SD, SEM of Δ-LP IN Group A and Group B

Statistical parameters	**Δ-LP of Group A (clobetasol) (mm)**	**Δ-LP of Group B (laser-PBM) (mm)**
Sample size	20	20
Mean	–0.0280	–0.0305
SD (standard deviation)	0.0443	0.0513
SEM (standard error of mean)	0.0099	0.0115

Chapter 6

DISCUSSION

OCT technology has evolved rapidly and substantially since the first evidence of skin imaging reported by Welzel et al in 1997. (61) OCT showed promising results on an extensive spectrum of inflammatory disorders, especially those defined by changes within the epithelium and dermo-epidermal junction, such as pemphigus, bullous pemphigoid and lichen planus (62) which can notoriously arise with coexisting signs in the oral cavity. As previously said, the aim 1 of this work was to assess OCT findings in the cheek of patients with established diagnosis of OLP, in order to determine the main differential features between disease-free mucosa and mucosa clinically affected by OLP. Such evaluation has been carried out with a newly-designed probe which, to our knowledge, was tested thus far for ex-vivo examination of nonmelanoma skin cancer and in recognizing healthy and pathological margins (63)Thus, the main strength of the present work relies on its novelty. Very few studies have investigated and described in such detail the OCT characteristics of atrophic-erosive Oral Lichen Planus (64), with no previous article exploring the potentialities of the dynamic scans of OCT in revealing the ultrastructural modifications of the epithelium and the underlying connective tissue. Concerning OCT, our experience suggested promising results as an additional device in investigating OLP, being a non-invasive tool with no biologic costs, capable of providing a complete scan in just thirty seconds. (65) However, the usage of OCT in our everyday clinical practice, lead to the identification of some limitations. For example, the necessity of an appropriate training and learning curve for OCT usage and interpretation of the scans. The latter is an issue that must be addressed, especially in oral medicine, considering the scattered evidence available in literature (66, 67), with scans coming from different OCT devices, pursued on different clinical entities, and no universally-accepted thesaurus available for the interpretation of OCT scan in this very field.

Our work revealed distinct ultrastructural differences between healthy controls and patients affected by OLP, with a very close agreement between OCT and histopathology scores, in terms of reduction of epithelial width and display of hyperparakeratosis, loss of integrity of basement membrane, and heavy inflammatory infiltration and increased vascularization within the lamina propria. (68) Further research should focus on the design of an oral probe with enough versatility to be applied in every mucosal district, ideally with a no-contact approach.

Larger samples will be then necessary to determine the sensitivity and specificity of such preliminary OCT findings in patients with atrophic-erosive Oral Lichen Planus.

Regarding aim 2, it consisted of the evaluation of morphometric changes of the oral tissues of patients with erosive and painful OLP undergoing laser-PBM (Group A), compared to drug topical steroid therapy (Group B).
According to our analysis, both PBM and topic clobetasol propionate were able to provide a significant modification of EP and LP width. (69,70,71) Regarding EP, an increase of the overall width was detected, suggesting the efficacy of both treatments in promoting the resolution of the epithelial atrophy, through a progressive restore of the epithelium turnover. On the contrary, a significant reduction of the LP area was detected, which instead might be indicative of the anti-inflammatory effect provided by PBM and clobetasol propionate, carried out as a temporary reduction of the band-like inflammatory cell infiltrate subjacent to the basal epithelial cells.
At the end of 8-weeks treatment, contrasting results were obtained: Group A exposed to clobetasol propionate seemed to experience a significantly higher increase of EP width (mean Δ-EP = 0.05 mm), when compared to Group B (Δ-EP = 0.02 mm), undergoing laser-PBM treatment.
On the other hand, this beneficial effect was not confirmed when analysis was focused on the corresponding variations of LP width, which displayed an overlapping behavior in the two groups, with a mean of reduction of 0.028 mm in Group A and 0.030 in Group B. Therefore, based on these preliminary OCT findings, it is not possible to clarify which treatment could be considered more efficacious, although clobetasol seemed to carry out a more satisfying restoration of the epithelium.
Such contradictory and unclear OCT findings can be somehow considered in line with the scarce clinical evidence available in literature regarding the differential effectiveness of topical corticosteroids compared to LLLT. A very recent systematic review by Akram et al. in 2018 (72) was the first study conducted to assess the efficacy of LLLT in comparison with corticosteroids in the treatment of OLP. According to the Authors, it was possible to consider as eligible only five studies. Of these, three studies revealed higher improvements with topical use of corticosteroids, one showed overlapping outcomes between the protocols, whereas one reported significant improvement of LLLT. However, it must be pointed out the overall low quality of evidence, with only three studies as RCTs, and a risk of bias described as high in four studies, and moderate in one, warranting the need of further randomized clinical trials.

Chapter 7

CONCLUSIONS

The present work was conducted to assess the potential uses of OCT in oral medicine, especially among patients affected by erosive-atrophic Oral Lichen Planus. Although oral biopsy remains the gold-standard for diagnosis of OLP, OCT played a promising role in monitoring the course of the disease, as well as the efficacy of treatment, by displaying a distinctive pattern of the disease and of its subsequent modifications after the exposure to clobetasol or PBM treatment. The main limitation of this study is the operator-dependent approach required for an innovative technique, since no probe for a thorough analysis of the oral cavity has been standardized yet, and the limited number of patients enrolled.
Further studies should focus on larger samples of patient, with the aim to understand if there could be an actual difference between clobetasol and laser treatment. Further research could be then focused on different entities commonly encountered in oral medicine in need of a constant follow-up, such as other premalignant disorders (e.g. proliferative verrucous leukoplakia, non-homogeneous leukoplakia), to follow-up patients with history of oral cancer, or to provide novel insights concerning rare autoimmune disorders, such as oral pemphigus and pemphigoid.

References

1. Sugerman PB, Savage NW, Walsh LJ, Zhao ZZ, Zhou XJ, Khan A, et al. The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med. 2002;13:350–65.

2. Scully C, Beyli M, Ferreiro MC, Ficarra G, Gill Y, Griffiths M, et al. Update on oral lichen planus: etiopathogenesis and management. Crit Rev Oral Biol Med. 1998;9:86–122.

3. Carrozzo M, Thorpe R. Oral lichen planus – a review. Minerva Stomatol. 2009;58:519–37.

4. van der Meij EH[1], van der Waal I. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. J Oral Pathol Med. 2003 Oct;32(9):507-12.

5. Cheng YS, Gould A, Kurago Z, et al. Diagnosis of oral lichen planus: a position paper of the American Academy of Oral and Maxillofacial Pathology. Oral Surg Oral Med Oral Pathol Oral Radiol 2016;122: 332–54.

6. Yang H, Wu Y, Ma H, et al. Possible alternative therapies for oral lichen planus cases refractory to steroid therapies. Oral Surg Oral Med Oral Pathol Oral Radiol 2016;121:496–509.

7. Regezi JA, Dekker NP, MacPhail LA, Lozada-Nur F, McCalmont TH. Vascular adhesion molecules in oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1996;81:682–90

8. Zhou XJ, Sugarman PB, Savage NW, Walsh LJ, Seymour GJ. Intra-epithelial CD8+ T cells and basement membrane disruption in oral lichen planus. J Oral Pathol Med. 2002;31:23–7

9. Redder CP, Pandit S, Desai D, et al. Comparative analysis of cell proliferation ratio in plaque and erosive oral lichen planus: An immunohistochemical study. Dent Res J (Isfahan) 2014;11:316–20.

10. Lo Muzio L, della Valle A, Mignogna MD, Pannone G, Bucci P, Bucci E, et al. The treatment of oral aphthous ulceration or erosive lichen planus with topical clobetasol propionate in three preparations: A clinical and pilot study on 54 patients. J Oral Pathol Med. 2001;30:611–7.

11. Beck HI, Brandrup F. Treatment of erosive lichen planus with dapsone. Acta Derm Venereol. 1986;66:366–7.

12. Arduino PG, Carbone M, Della Ferrera F, Elia A, Conrotto D, Gambino A, Comba A, Calogiuri PL, Broccoletti R. Pimecrolimus vs. tacrolimus for the topical treatment of unresponsive oral erosive lichen planus: a 8 week randomized double-blind controlled study. J Eur Acad Dermatol Venereol. 2014;28(4):475-82

13. Liu V, Mackool BT. Mycophenolate in dermatology. J Dermatolog Treat. 2003;14:203–11.

14. Hodak E, Yosipovitch G, David M. Low dose, low molecualr weight heparin (enoxaparin) is beneficial in lichen planus-a preliminary report. J Am Acad Dermatol. 1998;38:564–8.

15. Eisen D, Carrozzo M, Bagan Sebastian JV, Thongprasom K.14.Number V Oral lichen planus: clinical features and management. Oral Dis. 2005 ;11(6):338-49.

16. García-Pola MJ, González-Álvarez L, Garcia-Martin JM. Treatment of oral lichen planus. Systematic review and therapeutic guide. Med Clin (Barc). 2017 Oct 23;149(8):351-362.

17. Lundquist G, Forsgren H, Gajecki M, Emtestam L. Photochemotherapy of oral lichen planus. A controlled study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;79;554

18. Trehan M, Taylor CR. Low-dose excimer 308-nm laser for the treatment of oral lichen planus. Arch Dermatol 2004;140:415-20.

19. Aghahosseini F, Arbabi-Kalati F, Fashtami LA, Fateh M, Djavid GE. Treatment of oral lichen planus with photodynamic therapy mediated methylene blue: A case report. Med Oral Patol Oral Cir Bucal 2006;11:E126-9.

20. Van der Hem PS, Egges M, van der Wal JE, Roodenburg JL. CO_2 laser evaporation of oral lichen planus. Int J Oral Maxillofac Surg 2008;37:630-3.

21. Cafaro A, Albanese G, Arduino PG, Mario C, Massolini G, Mozzati M, *et al*. Effect of low-level laser irradiation on unresponsive oral lichen planus: Early preliminary results in 13 patients. Photomed Laser Surg 2010;28 Suppl 2:S99-103.

22. Walsh LJ. The current status of low level laser therapy in dentistry. Part 1. Soft tissue applications. Aus Dent J 1997;42:247-54.

23. Chung H, Dai T, Sharma SK, et al. The nuts and bolts of low-level laser (light) therapy.Ann Biomed Eng. 2012 ;40(2):516-33.

24. Caruso-Davis MK, Guillot TS, Podichetty VK, et al. Efficacy of low-level laser therapy for body contouring and spot fat reduction. Obes Surg. 2011;21(6):722-9.

25. Huang YY, Sharma SK, Carroll J, et al. Biphasic dose response in low level light therapy - an update. Dose Response. 2011;9(4):602-18.

26. Moraschini V, da Costa LS, Dos Santos GO. Effectiveness for dentin hypersensitivity treatment of non-carious cervical lesions: a meta-analysis. Clin Oral Investig. 2018 ;22(2):617-631.

27. Del Fabbro M, Corbella S, Sequeira-Byron P, et al. Endodontic procedures for retreatment of periapical lesions. Cochrane Database Syst Rev. 2016 Oct 19;10:CD005511.

28. Kellesarian SV, Malignaggi VR, Majoka HA, et al. Effect of laser-assisted scaling and root planing on the expression of pro-inflammatory cytokines in the gingival crevicular fluid of patients with chronic periodontitis: A systematic review. Photodiagnosis Photodyn Ther. 2017 Jun;18:63-77.

29. Dederich DN. Little evidence for the use of diode lasers as an adjunct to non-surgical periodontal therapy. Evid Based Dent. 2015 Mar;16(1):16.

30. Yan J, Zhang J, Zhang Q, et al. Effectiveness of laser adjunctive therapy for surgical treatment of gingival recession with flap graft techniques: a systematic review and meta-analysis. Lasers Med Sci. 2018 May;33(4):899-908.

31. He WL, Yu FY, Li CJ, et al. A systematic review and meta-analysis on the efficacy of low-level laser therapy in the management of complication after mandibular third molar surgery. Lasers Med Sci. 2015 Aug;30(6):1779-88.

32. Leung YY, Fung PP, Cheung LK. Treatment modalities of neurosensory deficit after lower third molar surgery: a systematic review. J Oral Maxillofac Surg. 2012 Apr;70(4):768-78.

33. Lin GH, Suárez López Del Amo F, Wang HL. Laser therapy for treatment of peri-implant mucositis and peri-implantitis: An American Academy of Periodontology best evidence review. J Periodontol. 2018 Jul;89(7):766-782.

34. Al-Maweri SA, Kalakonda B, AlAizari NA, et al. Efficacy of low-level laser therapy in management of recurrent herpes labialis: a systematic review. Lasers Med Sci. 2018 ;33(7):1423-1430.

35. Suter VGA, Sjölund S, Bornstein MM, et al. Effect of laser on pain relief and wound healing of recurrent aphthous stomatitis: a systematic review. Lasers Med Sci. 2017;32(4):953-963.

36. Al-Maweri SA, Javed F, Kalakonda B, et al. Efficacy of low level laser therapy in thetreatment of burning mouth syndrome: A systematicreview.Photodiagnosis Photodyn Ther. 2017 Mar;17:188-193.

37. Weber JB, Camilotti RS, Ponte ME, et al. Efficacy of laser therapy in the management of bisphosphonate-related osteonecrosis of the jaw (BRONJ): a systematic review. Lasers Med Sci. 2016 Aug;31(6):1261-72

38. Yousef M, Mansouri P, Partovikia M, Esmaili M, Younespour S, Hassani L. The Effect of Low Level Laser Therapy on Pemphigus Vulgaris Lesions: A Pilot Study. J Lasers Med Sci. 2017 Fall;8(4):177-180.

39. Pavlic V, Aleksic VV, Zubovic N, Veselinovic V. Pemphigus vulgaris and laser therapy: crucial role of dentists. *Med Pregl*. 2014;67(1-2):38-42.

40. Yilmaz HG, Kusakci-Seker B, Bayindir H, T.züm TF Low-level laser therapy in the treatment of mucous membrane pemphigoid. J Periodontol. 2010 Aug;81(8):1226-3

41. Cafaro A, Arduino PG, Broccoletti R. Low-level laser therapy for oral mucous membrane pemphigoid. Lasers Med Sci (2012) 27:1247–1250

42. Mutafchieva MZ, Draganova-Filipova MN, Zagorchev PI, Tomov GT. Effects of Low Level Laser Therapy on Erosive-atrophic Oral Lichen Planus. Folia Med (Plovdiv). 2018 Sep 1;60(3):417-424.

43. Agha-Hosseini F, Moslemi E, Mirzaii-Dizgah I. Comparative evaluation of low-level laser and CO2 laser in treatment of patients with oral lichen planus. Int J Oral Maxillofac Surg 2012;41(10):1265-9.

44. Zaid Hamdoon BDS, MSca, Waseem Jerjesa, TahwinderUpiled,g, Gordon McKenzied, Amrita Jayd, Colin Hopperb, Optical coherence tomography in the assessment of suspicious oral lesions: An immediate ex vivo study. Photodiagnosis and Photodynamic Therapy (2013) 10, 17—27

45. Olsen J, Holmes J, GEB Jemeca Advances in optical coherence tomography in dermatology—a review. Journal of Biomedical Optics 2018; 23(4), 040901.

46. Law TSM, Wu F, Xu H , Wang CC, Li TC. Endometrium imaging using real-time rotational optical coherence tomography imaging system. A pilot, prospective and ex-vivo study. Medicine (2019) 98:44

47. Attia ABE, Balasundaram G, Moothanchery M, Dinish US, Bi R, Ntziachristos V, Olivo M A review of clinical photoacoustic imaging: Current and future trends. Photoacoustics. (2019)7;16:100144.

48. Katkar RA, Tadinada SA, Amaechi BT, Fried D. Optical Coherence Tomography. Dent Clin North Am. 2018;62(3):421-434.

49. Walther J ,Schnabel C, Tetschke F, Rosenauer T, Golde J, Ebert N,Baumann M, Hannig C, Koch E, In vivo imaging in the oral cavity by endoscopic optical coherence tomography. J. Biomed. Opt. 23(7), 071207 (2018).

50. D. Ericson . Minimally invasive dentistry–concept and techniques in cariology," Oral Health Prev. Dent. 1(1), 59–72 (2003).

51. Sanda M, Shiota M, Imakita C, Sakuyama A, Kasugai S, Sumi Y.The effectiveness of optical coherence tomography for evaluating peri-implant tissue: A pilot study. Imaging Science in Dentistry 2016; 46: 173-8

52. Krause F, Schmalz G, Park KJ, Schmidt J, Ziebolz D, Schneider H, Haak R. Evaluation of calculus imaging on root surfaces by spectral-domain optical coherence tomography. Photodiagnosis Photodyn Ther. 2019 Mar;25:275-279.

53. Gentile E, Maio C, Romano A, Laino L, Lucchese A. The potential role of in vivo optical coherence tomography for evaluating oral soft tissue: A systematic review. J Oral Pathol Med. 2017;46:864–876

54. Walther J, Schnabel C, Tetschke F, Rosenauer T, Golde J, Ebert N,Baumann M, Hannig C, Koch E In vivo imaging in the oral cavity by endoscopic optical coherence tomography. J. Biomed. Opt. 23(7), 071207 (2018)

55. Jerjes W, Hamdoon Z, Yousif AA, Al-Rawi NH, Hopper C. Epithelial tissue thickness improves optical coherence tomography's ability in detecting oral cancer. Photodiagnosis Photodyn Ther. 2019 ;28:69-7

56. Paritosh Pande,a Sebina Shrestha,a Jesung Park,a Michael J. Serafino,a Irma Gimenez-Conti,b Jimi Brandon,b Yi-Shing Cheng,c Brian E. Applegate,a and Javier A. Joa Automated classification of optical coherence tomography images for the diagnosis of oral malignancy in the hamster cheek pouch. Journal of Biomedical Optics.2014; 19(8).

57. Heidari AE, Pham TT, Ifegwu I, Burwell R, Armstrong WB, Tjoson T, Whyte S, Giorgioni C, Wang B, Wong BJF, Chen Z The Use of Optical Coherence Tomography and Convolutional Neural Networks to Distinguish Normal and Abnormal Oral Mucosa. J Biophotonics. 2019 Nov 11

58. Duong S, Youssef J, Pimenta P, Aguigam H, Zhang J, Calantog A, Pilch S, Masters JG, Wilder-Smith P. An imaging-based approach to the evaluation of xerostomia. Lasers Surg Med. 2012 Aug;44(6):482-9.

59. Calantog A, Hallajian L, Nabelsi T, Mansour S, Le A, Epstein J, Wilder-Smith P. A Prospective Study to Assess In Vivo Optical Coherence Tomography Imaging for Early Detection of Chemotherapy- Induced Oral Mucositis. Lasers Surg Med. 2013 January ; 45(1): 22–27.

60. Di Stasio D, Lauritano D, Loffredo F, Gentile E, Della Vella F, Petruzzi M, LuccheseOptical coherence tomography imaging of oral mucosa bullous diseases: a preliminary study. Dentomaxillofac 2019 Aug 22:20190071.

61. Welzel J, Lankenau E, Birngruber R, Engelhardt R. Optical coherence tomography of the human skin. J Am Acad Dermatol. 1997 Dec;37(6):958-63.

62. Olsen J, Holmes J, Jemec GB Advances in optical coherence tomography in dermatology-a review. J Biomed Opt. 2018 Apr;23(4):1-10.

63. Rashed D, Shah D, Freeman A, Cook RJ, Hopper C, Perrett CM. Rapid ex vivo examination of Mohs specimens using optical coherence tomography. Photodiagnosis Photodyn Ther. 2017 Sep;19:243-248

64. Fomina IuV, Gladkova ND, Leont'ev VK, Urutina MN, Gazhva SI, Snopova LB, Gelikonov VM, Kamenskiĭ VA. Optical coherence tomography in the evaluation of the oral cavity mucosa. Part II. Benign and malignant diseases. Stomatologiia (Mosk). 2004;83(4):25-32.

65. Prestin S, Rothschild SI, Betz CS, Kraft M. Measurement of epithelial thickness within the oral cavity using optical coherence tomography. Head Neck. 2012;34:1777-1781.

66. Green B , Cobb AR, Brennan PA, Hopper C.Optical diagnostic techniques for use in lesions of the head and neck: review of the latest developments. Br J Oral Maxillofac Surg. 2014 Oct;52(8):675-80.

67. Wei Wei, Woo June Choi, Ruikang K. Wang. Microvascular imaging and monitoring of human oral cavity lesions in vivo by swept-source OCT based angiography. Lasers Med Sci. 2018 January ; 33(1): 123–134.

68. Ianoși SL, Forsea AM, Lupu M, Ilie MA, Zurac S, Boda D, Ianosi G, Neagoe D, Tutunaru C, Popa CM, Caruntu C. Role of modern imaging techniques for the in vivo diagnosis of lichen planus. Exp Ther Med. 2019 Feb;17(2):1052-1060.

69. Anitua E, Piñas L, Alkhraisat MH. Histopathological features of oral lichen planus and its response to corticosteroidtherapy: A retrospective study.Medicine (Baltimore). 2019 Dec;98(51):e18321.

70. Ferri EP, Gallo CB, Abboud CS, Yanaguizawa WH, Horliana ACRT, Silva DFTD, Pavani C, Bussadori SK, Nunes FD, Mesquita-Ferrari RA, Fernandes KPS, Rodrigues MFSD. Efficacy of photobiomodulation on oral lichen planus: a protocol study for a double-blind, randomised controlled clinical trial. BMJ Open. 2018 Oct 8;8(10):e024083

71. Arduino PG, Campolongo MG, Sciannameo V, Conrotto D, Gambino A, Cabras M, Ricceri F, Carossa S, Broccoletti R, Carbone M. Randomized, placebo-controlled, double-blind trial of clobetasol propionate 0.05% in the treatment of oral lichen planus. Oral Dis. 2018 Jul;24(5):772-777.

72. Akram Z, Abduljabbar T, Vohra F, Javed F. Efficacy of low-level laser therapy compared to steroid therapy in the treatment of oral lichen planus: A systematic review. J Oral Pathol Med. 2018 Jan;47(1):11-17.